THE NUTRITIVE PROPERTIES OF THE SWEET POTATO

BY

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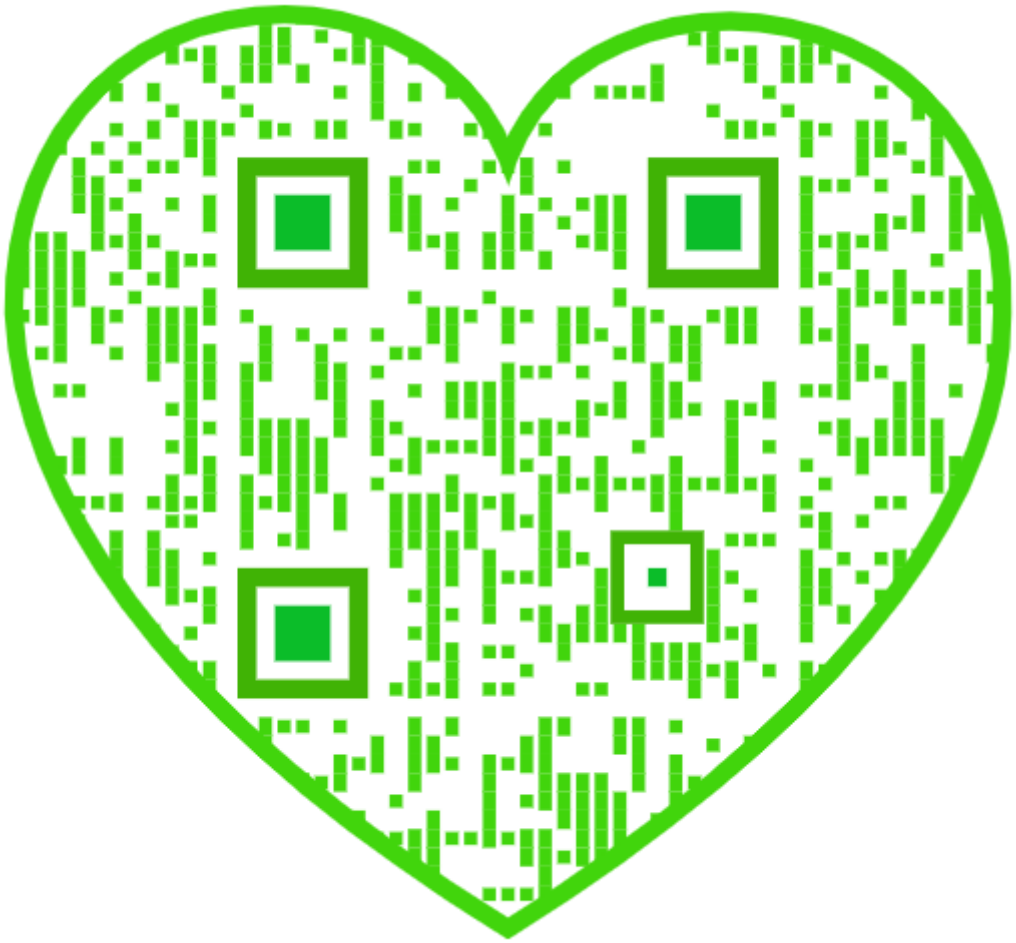

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Margaret Hatfield

ENTITLED "The Nutritive Properties of the Sweet Potato"

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Home Economics

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I.

INTRODUCTION

The study of nutrition has long been of interest and still offers many unexplored fields for research. As knowledge of chemistry, physiology and other sciences increased, however, the emphasis was placed on different phases of the problem and the measuring unit changed continually. Even before 1780 there were many investigators who felt a deep interest in the subject, but it is now conceded that the originator of our conception of life--and hence nutrition--as a chemical process was Lavoisier. His renown brought young scientists from other countries into his sphere of influence and it is undoubtedly true that Voit, and through him Rübner, Fischer and many others owed their initial inspiration to this one man. His influence may be traced directly thru most of the achievements in physiological chemistry in the nineteenth century.

A. CALORIC VALUE OF FOODS.

The investigations in the field of respiration by Carl von Voit, and the calorimeter constructed and tested by him and by Pettenkofer in 1866, mark the beginning of an interest in the heat values of various compounds, for the body's use. From then on until the beginning of the twentieth century the emphasis came to be placed more and more on the caloric requirement of the body.

Rübner's isodynamic law, the first conception of which was due to Voit, was formulated in 1885. This law, "that foodstuffs may under given conditions replace each other in accordance with their heat-producing value" was carefully verified in 1901 when the value of 4.1 large calories per gram of protein and carbohydrate, and 9.3 per gram of fat, now generally accepted were restated. Perhaps the construction of the Atwater-Rosa calorimeter and its perfection in 1897 may be taken as representing the highest point in the development of the study of the caloric value of foods.

B. PROXIMATE COMPOSITION OF FOODS.

During the latter part of the nineteenth century an interest in what are generally termed the "proximate" components of food had been developed at the same time as that which saw the caloric determinations being made. Comparatively few analyses of foods were made before 1893, but the exhibits of foods from all over the world at the World's Fair suggested an unusual opportunity for procuring samples for analysis. The work of analyzing these samples was carried on by the Bureau of Chemistry at Washington and by Atwater at Middletown. The first edition of Atwater's "Chemical Composition of American Food Materials" in 1896 was a significant contribution to food analysis, a contribution made more valuable by its revision in 1906.

C. STANDARDS OF DIET.

The relation of the proximate constituents of a food to metabolism was early recognized. The much discussed protein al-

lowance of Voit, 118 grams per day for a man doing a moderate amount of work, remained as the standard for a normal diet until Siven, in 1901, discovered the possibility of lowering the intake of protein to about one-fourth that amount. In 1904 Chittenden substantiated this possibility, setting the protein standard at 40 grams per day and the caloric intake at 2000, a figure much lower than had previously been considered necessary. The caloric requirement as given by other investigators is:

Voit	3055 calories
Rübner	2868 calories
Atwater	3400 calories

All the figures are those for a man of average weight performing a moderate amount of work. The question is still open, as is that of protein requirement, but many workers, Lusk and Atwater for example, consider the Chittenden level too low.

The problem of the effect of the proximate composition of the food on metabolism developed several complex phases. A survey of the literature of a decade ago shows that the interest was concentrated on at least two phases; the fat content of the diet and the amino acids making up the proteins. As both lines of research formed a background to the later investigations of vitamines, a brief summary of these subjects seems appropriate.

D. THE VALUE OF CERTAIN AMINO ACIDS.

The question of the part played by amino acids in nutrition is one concerning which there has been much investigation. Emil Fischer's discovery of the amino acids and his analyses of certain proteins, such as gelatine, in 1902, gave an impetus to other investigators. In 1911 Osborne and Mendel[1-2] showed that there was a marked difference in the nutritive power of certain proteins. Casein and zein, for example, gave very divergent effects, when each was used as the sole protein in a diet. This inequality in the efficiency of the proteins was early shown by these workers[3] to lie in the fact that some contained certain specific amino acids essential to nutrition--tyrosine, tryptophane and others,--while some proteins did not contain these amino acids. It was in the study of the amino acids that our conception of nutrition was enlarged to include maintenance, growth and reproduction. The requirements of protein alone for these three phases of nutrition varied greatly. For example, tryptophane was able to supplement the deficient proteins of corn[4] for a maintenance

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1. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1911-12, 11, xxii.
2. Osborne, T. B., and Mendel, L. B.: Science, 1911, 34, 722.
3. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1912, 12, 473.
4. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1913, 14, xxxi.

of body weight, but the investigators found that in order to obtain growth as well as maintenance, lysine must be included in the protein intake.[5] Reproduction will not be discussed in this place as the experiments to be described did not include that phase of nutrition. A comparison of some of the proteins which contain the essential amino acids[6] showed that a different intake was necessary for growth, depending on the proportions of the essential amino acids present in the proteins.

The experiments by which Osborne and Mendel established these theories were carried out on white rats. The rats were fed a standard diet in which the variable factor was the type of protein used. The diets in each case, however, were supplemented with "protein-free milk" which, as first prepared, contained milk sugar, inorganic salts and "other water-soluble constituents of milk". Attention is called in one of the Osborne and Mendel papers[7] to the fact that in every case where isolated proteins, adequate for maintenance were used, the animal was kept in health by the use of "protein-free milk", while with a pure salt mixture the animal showed signs of malnutrition. In the light of more recent knowledge of nutrition it is plain that vitamines were supplied to the diet with the "protein-free milk". The workers recognized

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5. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1914, 17, xxiii.

6. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1915, 20, 351.

7. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1912-13, 13, 233.

that some such substance was present in "protein-free milk"[8] and in later experiments, after the existence of vitamines was estaclished, a synthetic salt mixture was used to eliminate complications. They stated,[9] however, that the presence of vitamines in the salt mixture was immaterial in determinations of protein minima and similar values.

E. THE WATER-SOLUBLE VITAMINES.

The occurrence of the water-soluble vitamine in "protein-free milk" by no means marks the first appearance of such substances as complicating factors. Beri-beri, polyneuritis and scurvy are among the more commonly recognized deficiency diseases and the literature on all three types is extensive. For years the deficiency diseases were recognized, while the explanations of the underlying causes were numerous and far from being in agreement. In 1910 Schaumann[10] induced an experimental beri-beri in dogs by feeding them meat previously boiled with soda. He attributed the disease to the breaking of an organic phosphorus linkage in the meat, brought about by the alkali. Funk[11] isolated a substance from rice polishings which would cure beri-beri or polyneuritis in

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8. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1913, 15, 311.

9. Osborne, T. B., and Mendel, L. B.: Biochem. J., 1916, 10, 534.

10. Schaumann, H.: Archiv f. Schiffs-u. Tropennyg; 1910, 14, Beiheft 8.

11. Funk, C.: J. Physiol., 1911, 43, 395.

birds, and which he believed to be an organic base. His method for procuring this organic base involved precipitation with phosphotungstic acid. In this manner he prepared a substance carrying the vitamine from rice polishings, or milk,[12] minimal amounts of which cured the polyneuritic pigeons. Another method of obtaining the antineuritic vitamine in combination is that of Seidell[13] who found that it was adsorbed almost quantitatively by fuller's earth. McCollum[14] used dextrin as an adsorbent medium for the vitamine. The literature on scurvy can only be touched upon in this paper,[15] but the few references cited above go to prove that the water-soluble vitamines constitute a fertile field for research.

F. THE PLACE OF FATS IN THE DIET.

Another phase of nutrition, the study of which yielded unexpected results, was that concerning the place of fats in the diet. Just as the study of protein and its importance in nutrition led to the discovery of the water-soluble vitamines, so a study of the rôle played by fats resulted in the subsequent discovery of another type of vitamine. As early as 1909 William Stepp[16] published data to show that white mice were able to live

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12. Funk, C.: J. Biol. Chem., 1912, 45, 75.

13. Seidell, W.: U. S. Pub. Health Reports, 1916, No. 325.

14. McCollum, E. V.: J. Biol. Chem., 1918, 33, 55.

15. For a complete bibliography on beri-beri, polyneuritis and scurvy see Eddy, W. H.: Abstracts of Bacteriology, 1913, 3, 313: also Blunt, K., and Wang, C. C.: J. Home Ec., 1920, 12, 1.

16. Stepp, W.: Biochem. Z., 1909, 22, 452.

on a diet of milk-bread, unless the ration were previously extracted with ether and alcohol. He believed that after such extraction failure to grow and subsequent death were due to the absence of "lipoids" in the diet. Since there seemed to be some question as to whether the extracted substances were lipoids or true fats, Stepp's further work[17-18-19] was undertaken to settle this point. He was able to show that fats as such were quite dispensable. On the other hand if the lipoids had been removed by extracting the mixture with alcohol, the animals died. For these reasons he believed the indispensable factor to be a lipoid. That true fats are entirely dispensable was proved also by Osborne and Mendel[20]. Their conclusions disagreed, however, with Stepp's as to the need of lipoids in the diet. The great efficiency which they found for butter-fat and egg yolk[21] was in marked contrast to the lack of nutritive properties in olive oil and lard. Since the latter substance contains more cholesterol and lecithins than butter, this investigation seemed to show that the presence of lipoids in the diet was less important than Stepp's observation would lead one to believe. In 1913 McCollum and Davis[22] published their data on the

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17. Stepp, W.: Z. Biol., 1911, 57, 135.

18. Stepp, W.: Ibid, 1912, 59, 366.

19. Stepp, W.: Ibid, 1913, 62, 405.

20. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1912, 13, 81.

21. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1913, 16, 423.

22. McCollum, E. V., and Davis, M.: J. Biol. Chem., 1913, 15, 167.

question of the indispensable nature of lipoids in the diet. From this data they concluded that resumption of growth after failure to grow when lipoids were absent in the diet was due either to the addition of certain lipoids or of substances carried with the lipoids. From this view of the matter it was a short and natural step to the conception of the so-called "fat-soluble A". Lipoids alone had proved incapable of supplementing an alcohol-extracted diet. Some substance carried with the lipoids gave definite, beneficial results when used with such a diet, altho the cause of these results was not known. The question of isolating and identifying this substance remained. Because of the one characteristic which was then recognized, its solubility in fats, it was called the "fat-soluble vitamine" or "fat-soluble A".

G. SOURCES OF THE FAT-SOLUBLE VITAMINE.

While this vitamine was called "fat-soluble", for the above reasons, further investigations soon showed only certain of the natural fats and oils as sources of the vitamine. Osborne and Mendel found it present in butter,[23] egg-yolk[23] and in cod-liver oil.[24] In contrast to these results other fats such as lard,[25] olive oil,[25] and almond oil,[26] had no effect in improving the sore

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23. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1913, 16, 423.

24. Osoorne, T. B., and Mendel, L. B.: J. Biol. Chem., 1914, 17, 401.

25. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1913, 16, 423.

26. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1914, 17, 401.

eyes and debilitated conditions which always resulted from a lack of the fat-soluble vitamine. Osborne and Mendel also found[27] that this vitamine was present in greater concentration in the oil fraction of the various fats than in any other part. McCollum and Davis[28] showed that vegetable fats such as corn oil, and the fat in the wheat embryo were as capable of supplying the fat-soluble vitamine as the animal fats.

It seems remarkable, as shown even in so brief a review of the literature, that the results obtained in early investigations were in such close agreement. Altho there has since been some discussion as to the identity of the fat-soluble vitamine--discussed later in relation to the question of pigment (page 14)--the original conception of its functions remains substantially as it was first formulated.

H. TYPES OF VITAMINES.

The many experiments of recent years have defined quite sharply the functional limitations of at least three specific vitamines, now known as "fat-soluble A", "water-soluble B"--regarded by some investigators as identical with the antineuritic vitamine--and the antiscorbutic vitamine.

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27. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1915, 20, 379.

28. McCollum, E. V., and Davis, M.: J. Biol. Chem., 1915, 21, 179.

I. CHEMICAL NATURE OF VITAMINES.

Many investigations have been carried on by Funk, Williams, Seidell, Steenbock and others to determine the chemical nature of the vitamines. It has proved impossible, up to the present time to obtain any of the vitamines in a pure state, and for that reason the chemical structure is still unknown.

J. THE IMPORTANCE OF VITAMINES.

While much remains to be discovered concerning vitamines, their importance in nutrition is an established fact. Altho it is recognized that our knowledge of proteins, salts and calories must be increased before much can be learned of the vitamine content of a given food, the interest still centers on the latter point. Because of the rapid and efficacious results obtained by the addition of vitamines to any dietary, particularly that of a child, it is to be hoped that further work will soon result in the obtaining of pure vitamines.

II.

THE SWEET POTATO

A. REASONS FOR CHOOSING THE SWEET POTATO.

The experiments to be described in this paper were undertaken in an attempt to discover the adequacy or specific deficiency of the nutritive qualities of the sweet potato. This root was chosen for a number of reasons.

1. Wide Use.

The sweet potato is widely used in this country. It is particularly common in the southern and central districts where the cold is not intense enough to cause the freezing of the potatoes.

2. Large Annual Production.

The annual production in this country is so large that the sweet potato constitutes an important staple article of food. In 1917-18,[29] with deductions made for spoilage and other loss, the crop was estimated at something over fifty-eight million bushels.

3. Availability as a Substitute for Other Starchy Foods.

The large production of sweet potatoes in this country and the fact that the sweet potato contains so large a percentage

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29. Pearl, R. "The Nation's Food", Philadelphia, 1920.

of starch suggested the possible availability of the sweet potato as a substitute for other starchy foods. The habit of substitution, carried over from war-time, makes this a question of popular interest.

4. Uncertainty as to Digestibility.

The ease of digestibility of the sweet potato has long been in question, though there has been little proof that digestion was retarded by its presence in the diet. The prevalence of this idea was one of the important reasons for the use of the sweet potato in these experiments. Altho it does not always follow that the physiological reaction of the rat is the same as that of man, the results with rats are in general applicable to man.

B. LITERATURE.

1. Roots and Tubers.

The literature contains few references to the nutritive value of roots and tubers. An article by Denton and Kohman[30] discusses the carrot, and McCollum, Simmonds and Parsons[31] have worked with the Irish potato. Except for these papers no work was published on this type of vegetable prior to the time the present experiments were undertaken. Since that time Steenbock

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30. Denton, M. C., and Kohman, E.: J. Biol. Chem., 1918, 36, 249.

31. McCollum, E. V., Simmonds, N., and Parsons, M.: J. Biol. Chem., 1918, 36, 197.

and Gross[32] have reported in some detail the dietary availability of most of the common roots and tubers. In this paper they have shown that the peeled and dried sweet potato is available in amounts up to 60% of the diet.

2. "Fat-Soluble A" and Plant Pigments.

Reference has already been made to the discovery of the fat-soluble vitamine. Steenbock's theory[33] of the association of this vitamine with plant pigments as it was first outlined seemed convincing. He found that rats fed on yellow corn as the sole source of "fat-soluble A" thrived, while those fed on white corn showed all the ordinary symptoms usually present when that vitamine is absent from the diet. Palmer,[34] however, has shown that the fat-soluble vitamine is certainly not identical with the carotinoids, and Steenbock[35] now states that, altho the vitamine has been associated with pigment in various cases, "it is not carotin". The Palmer[34]-Steenbock[35-36] controversy, which has arisen since the beginning of this set of experiments, lent an added interest to the subject in hand because of the presence of considerable pigment in the sweet potato.

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32. Steenbock, H., and Gross, E. G.: J. Biol. Chem., 1919, 40, 501.

33. Steenbock, H.: J. Biol. Chem., 1920, 41, 81.

34. Palmer, L. S.: Science, 1919, 50, 501.

35. Steenbock, H.: J. Biol. Chem., 1920, 41, xii.

36. Steenbock, H.: Science, 1919, 50, 352.

C. CHEMICAL COMPOSITION OF THE SWEET POTATO.

Altho the nutritive value of the roots and tubers had been comparatively neglected, certain facts were known regarding their composition. The average composition of the sweet potato, as given by Atwater and Bryant,[37] is as follows:

Water	69. %
Protein	1.8%
Fat	.7%
Total Carbohydrates	27.4%
Ash	1.1%

A consideration of these figures showed at once that even if the quality of the protein should prove adequate, the amount present in the sweet potato would be insufficient for growth. The mineral content is also low, and it seemed reasonable to suppose that additions of minerals and protein would be necessary in a diet of sweet potatoes. Nothing was known, however, of the vitamine content at the time the experiments reported in this paper were undertaken.

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37. Atwater, W. O., and Bryant, A. P.: Dept. of Agriculture, Office of Experiment Stations, Bulletin 28, 1906.

III.

OBJECT OF EXPERIMENTS

The object of these experiments was to examine the nutritive properties of the sweet potato in respect to its content of protein, minerals, "fat-soluble A" and "water-soluble B", and to determine the physiological effects of a diet composed largely or wholly of sweet potatoes.

IV.

EXPERIMENT

A. GENERAL PROCEDURE.

The following procedure is now generally accepted as standard in estimating the nutritive properties of foods. The original food is so prepared as to furnish a uniform product which has not lost any physiological properties, which will not deteriorate and which can be made in relatively large quantities. Rapid drying at low temperatures is perhaps the most common manner of treating vegetables, as this effects little change in the nutritive properties and the dried product is not as susceptible to spoilage as the original. The product so obtained is often tested against the fresh vegetable to see if the nutritive properties have been altered in the process of preparation.

B. METHOD.

Some experimental animal is used, preferably a small one, as the equipment for caring for small animals is not expensive and the food intake is relatively small.

In general, the first diet is one consisting solely of the food under consideration. The next diet consists of that food with the addition of some one substance which insures the adequacy of the food in one respect. The diets are made more and more com-

plex until all possible combinations of food and adjuncts have been tried. A "complete" protein, a carbohydrate, a salt mixture and substances carrying the vitamines comprise the usual adjuncts. These are prepared in as pure a state as possible and special precautions are taken to avoid the contamination of purified proteins with vitamines or inorganic salts. The fat content of the diet is not considered particularly important, as the calorific requirement may be made up with carbohydrates. Lard, however, is often used to increase the caloric value and also to bind the mixtures together.

C. METHOD USED IN PRESENT EXPERIMENT.

For the present work the albino rat was taken as the experimental animal. The diets fed consisted in part or in whole of sweet potato which had been dried in a current of air in a warm room. Casein as the supplemental protein, butter-fat as the source of fat-soluble-A, dried brewers' yeast as the source of water-soluble-B, and a synthetic milk-salt mixture were used as the adjuncts. Since distilled water made a satisfactory paste, and the caloric value of the food was relatively high, no lard was used in the mixtures. Every attempt was made to have the supplementary substances as pure as possible.

1. Preparation of Supplementary Substances.

a. Casein:--The casein was prepared in two ways. For part of the experiment, a modification of the Van Slyke, Bosworth[38]

38. Van Slyke, L., and Bosworth, G.: J. Biol. Chem., 1913, 14, 204.

method was used. The casein was precipitated from skim milk by dilute acetic acid, (6 c.c. per liter); dissolved in dilute NH_4OH, (6 c.c. per liter), and filtered thru cotton. The process was repeated five or six times. After the last precipitation the casein was washed with distilled water until free from chlorides, and sucked as dry as possible on a Buchner funnel. It was then triturated twice with 95% alcohol, and refluxed for twelve hours with absolute alcohol. The alcohol was removed by suction, and the casein pressed between sheets of filter paper and dried in an electric oven at a temperature below 60° C. The product was a white powder, presumably free from water, salts and vitamines.

There is some question as to the necessity of the long refluxing process. Hopkins[39] and Funk[40] found that very small amounts of milk contained a relatively large proportion of water-soluble-B. This point was first questioned by Osborne and Mendel[41] in 1918. Their deductions were so much at variance with those of the earlier writers that it was considered advisable, at the time this work was begun, to reflux the casein with absolute alcohol in order to effect the complete removal of any water-soluble-B; and

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39. Hopkins, F. G.: J. Physiol., 1912, 44, 425.

40. Funk, C.: Biochem. J., 1913, 7, 211.

41. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1918, 34, 537.

this was carried out for each lot of casein to insure uniform results. A later article by Osborne, Wakeman and Ferry[42] showed that even crude casein contains little or no water-soluble-B, and Osborne and Mendel[43] have since published data which prove that milk itself contains very little of that vitamine.

The method described above proved inadequate for making large amounts of casein with the facilities at hand. Hence another process was used for most of the experiment. Commercial casein, Baker's, was washed with dilute acetic acid and with distilled water for seven or eight days, according to the method used by McCollum.[44] It was then triturated twice with 95% alcohol, refluxed for twelve hours with absolute alcohol, and dried. There seemed to be no difference, physiologically, between the caseins prepared in these two ways. Steenbock[45] found cold 95% alcohol an excellent solvent for fat-soluble-A, so the double trituration as well as the long refluxing were counted on to remove it. Enough casein was added to the ration to bring the total protein to 18%.

b. Butter-fat:--The butter-fat was prepared by melting

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42. Osborne, T. B., Wakeman, A., and Ferry, E. L.: J. Biol. Chem., 1919, 39, 35.

43. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1920, 41, 515.

44. McCollum, E. V.: J. Biol. Chem., 1915, 23, 231.

45. Steenbock, H.: J. Biol. Chem., 1920, 42, 131.

fresh unsalted butter at 60° C. and filtering thru a jacketed funnel. It formed 5% of the diet.

c. Salt Mixture:--The salt mixture was made up according to the Osborne and Mendel[46] formula and was added as 4% of the diet.

d. Yeast:--The yeast was supplied by a Chicago brewing company.* For a few experiments .5% was used, but this was soon increased to 2%.[47]

2. Preparation of Sweet Potato.

In preparing the sweet potatoes an attempt was made to hasten the process of drying and at the same time to prevent a loss or depletion of vitamines due to overheating. Virginia sweet potatoes, all from the same shipment were thoroly washed and decaying portions removed. They were then ground in a meat chopper, spread in thin layers on large trays, and dried under an electric fan at a temperature of 35° C. The dry product was ground to a powder in a corn mill.

Starch was not added to the diet as the sweet potato has a sufficiently high starch content to furnish adequate carbohydrates.

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46. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1919, 37, 572.

* We are indebted to the Schoenhofer Brewing Co. for the dried yeast used in these experiments.

47. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1917, 31, 149.

3. Care of Experimental Animals.

The rats were kept in wire cages set on pans of sawdust, the cage being large enough to permit of exercise. Shredded paper was used for bedding, and the paper and sawdust were changed once a week or oftener if necessary. Young rats of 50-60 grams were used for all except the first set of experiments, and when put on the various diets were uniformly slender, active and eager for food. Food was offered to the experimental animals in heavy glass sponge cups which they could not upset. More food than they could use was given at each feeding. Only small amounts of food were left over, and this residuum was not weighed. Glass dishes were chosen as being easily cleaned, heavy, and of a suitable shape to prevent scattering of food. Distilled water was used exclusively for the rats on experimental diets.

The breeding rats were fed a mixed diet of milk, grains and a dog-biscuit. The litters, after weaning, were given the same food until 50-60 grams in weight, when they were put on a special diet. The experimental animals were segregated as the time available did not suffice for the rearing of young from these rats. In only one case [Chart 15] did a female on experimental diet give birth to a litter. The young all died or were born dead, and the mother devoured most of them. Since it is a generally accepted fact that young rats will often kill and eat their first litter, the eating of the offspring in the above case may be attributed to the age of the mother (97 days) rather than to the diet which had proved fully adequate for growth. One of the rats

on a normal diet (92 days old), which was being used as a control, acted in much the same manner with her first litter, though the next litter was entirely normal and healthy.

In order to show the inadequacies of the different diets, the rats were weighed twice a week. Changes in appearance were noted daily, particularly the condition of the hair, ears, nose and tail, any defects in posture, or signs of decreased vitality. The gait of the rat as it crossed the pen was often as indicative of malnutrition as any other symptom. Dead animals were weighed and then autopsied, to detect seepage from the intestines, the presence of pleural fluid, and underdevelopment of the generative organs.

V.

RESULTS

A. EXPERIMENTAL OBSERVATIONS.

1. Effect of Diets on Growth.

The control rats were kept on a mixed diet. Their most rapid growth occurred during the first thirteen or fourteen weeks of life, and after that time there was slower but continued gain in weight [Chart 1] . For the first set of experiments animals weighing about 40 grams were given sweet potato as the sole source of protein, salts, vitamines and energy. The rats declined in weight immediately, and all died within 30-50 days. Later on, older rats weighing about 100 grams were put on the same diet. The contrast in results was marked. There was a sharp drop in weight during the first week, after which the decline was so slow as to approximate maintenance in one case. [Rat 27, Chart 2.] One of the rats died after being on a sweet potato diet for 65 days. Another was killed at the end of eleven weeks because of his emaciated and feeble condition. The slower decline of the older rats may be explained by the fact that they possessed greater reserves of body tissue upon which to draw. All the rats on a diet limited to sweet potato showed the same symptoms just before death. They were greatly emaciated, with rough hair, sunken and usually sore eyes, a scabby nose, ringed tail, very pale ears, and the character-

istic hump-backed gait of intestinal disorders. In addition, they suffered from a destruction of lung tissues, and in the older rats whose life was so much prolonged, there was very little of the lung tissue left, but much pleural fluid. Rat 27 had the erected penis mentioned by Denton[48] for two weeks before he was killed. The generative organs were undeveloped.

Another set of rats was kept on a sweet potato diet for three weeks, during which time there was steady decline in weight. Four were then given casein with the sweet potato, and two others were put on a normal mixed diet. [Chart 3]. With the addition of casein the growth was much better for a time, but the growth on this diet was not as rapid as that shown by the rats which had been put back on a normal diet. This seemed to show that protein was a necessary supplement to the sweet potato but not the only one.

Rats whose diet during the whole period consisted of sweet potato and casein maintained their weight and even made slight gains, but there was not normal growth. [Chart 4].

The addition to a sweet potato diet of salts, butter or yeast, singly or in all the possible combinations [Charts 5, 6, 7, 8, 9, 10], gave the same type of curve as the diet of sweet potato alone. In this series the diets without yeast seemed a little less adequate than the others [Chart 9], possibly due to the fact that the yeast proteins may have acted as supplements in diets

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48. Denton, M., and Kohman, E.: J. Biol. Chem., 1918, 36, 249.

including this adjunct. The symptoms of distress were most marked in diets containing neither yeast nor casein. That the yeast proteins are from 76-83% available in rat nutrition has been shown by Osborne and Mendel.[49]

The foregoing experiments indicated the need for the addition of adequate proteins to a sweet potato diet. As normal growth was not obtained with casein and sweet potato alone, other combinations were tried.

A diet of sweet potato, casein and yeast [Chart 11], proved no more adequate than the mixture of sweet potato and casein, suggesting that it is not water-soluble-B in which the sweet potato is deficient.

The supplementing of sweet potato and casein with butter [Chart 12] or butter and yeast [Chart 13] gave somewhat better curves. That this result was not due to any lack of fat-soluble-A in the potato is shown by later experiments [Chart 15]. That the improved growth was not due to the substitution of a high calorie diet for one low in calories is shown by the fact that normal growth was obtained on a diet containing no added fat [Chart 14]. It seems probable that the slight improvement in growth may have been due to an increased palatability of the mixture.

With sweet potato, casein and salts [Chart 14] a normal growth curve was obtained, while the addition of butter [Chart 15], yeast [Chart 17] or butter and yeast [Chart 16] to this diet did

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49. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1919, 38, 223.

not improve it. In fact, the curves were a little below normal when other materials were added to the "complete" diet of sweet potato, casein and salts.

2. Clinical Symptoms.

Observations of the clinical symptoms of the rats on inadequate diets constituted the basis for determining the specific inadequacies of the food.

a. Sore Eyes:--Investigators have been accustomed for some time to associate the soreness of the eyes known as xerophthalmia with the state of malnutrition resulting from a lack of "fat-soluble A". Bulley,[50] on the contrary, makes a definite distinction between real xerophthalmia and the condition brought about by the absence of the fat-soluble vitamine in the diet. She states that this latter result is due to unsanitary conditions in the care of the animals and an increased susceptibility to infections as the result of malnutrition. She also claims that the condition may be completely cured, with boracic acid treatments. Drummond[51] has remarked that with an inadequate supply of "fat-soluble A" there is a lowering of resistance to disease, but an absence of characteristic pathogenic lesions. This is confirmed by Emmet and Luros.[52] On the other hand, Harden and Zilva[53] and Osborne and Mendel[54]

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50. Bulley, E. C.: Biochem. J., 1919, 13, 103.

51. Drummond, J. C.: Biochem. J., 1919, 13, 95.

52. Emmet, A. D., and Luros, L.: J. Biol. Chem., 1920, 41, liii.

53. Harden, A., and Zilva, S. S.: Biochem. J., 1918, 12, 408.

54. Osborne, T. B., and Mendel, L. B.: J. Biol. Chem., 1920, 41, 549.

found that xerophthalmia always occurred on a diet free from this vitamine, even when the conditions were most sanitary. The latter authors suggest that Bulley is referring to keratomalacia, or xerotic keratitis.

In the experiments with sweet potato the growth curves indicated no lack of fat-soluble-A, but it has been a very common thing for sore eyes to develop among the rats on diets deficient in protein. With one exception, all the rats on a diet of sweet potato alone had developed a soreness of the eyes at the end of the period. In fact the sore eyes usually were in evidence several weeks before death occurred. This was not xerophthalmia, however, as the eyes were sunken rather than protruding, and showed a conjunctivitis. Typical xerophthalmic rats show a slightly bulging eyeball, with the lids drawn almost shut over it. No cases of xerophthalmia developed in any of these experiments. Hence, one of Bulley's premises, that malnutrition induces susceptibility to infection of the eyes, has been substantiated in these experiments. As the animals were kept under highly sanitary conditions, and none of them showed evidences of parasites, that factor can be eliminated from a consideration of the results obtained.

b. Bloating:--Bloating is of common occurrence where there is a lack of adequate proteins. The so-called war-edema found in some of the devastated districts of Europe and the Near East after the war is a concrete illustration, on a large scale, of this fact, tho the diets causing this edema were lacking in other substances

besides protein. Denton[55] has described a bloating or dropsy which occurred on a too-limited diet of carrots.

When sweet potato was used as the sole source of protein in the diet, there was a great deal of bloating, especially on the diet of sweet potato and salts. In one of the rats [Number 48, Chart 5] a real edema developed over the head, chest and abdomen. On autopsy the infiltrated tissue over the sternum proved to be one-quarter of an inch thick, and jelly-like in consistency. Other rats developed an edematous condition over the head and sternum at one time or another, and considerable bloating of the peritoneum and abdomen, but in no others was it so extreme a condition as in the case cited. As has been said before, the conditions were slightly better when yeast was part of the casein-free diet, probably due to the proteins of the yeast. The frequent bloating described by Steenbock[56] on a diet of 60% sweet potato, made nearly adequate by the addition of suitable protein, salts, etc., was not observed in cases where the sweet potato formed 72-80% of the diet. Bloating was always accompanied by large amounts of peritoneal fluid.

c. Lung Complications:--In numerous cases, particularly on inadequate-protein diets, the lungs were pale, infarcted or partly destroyed, and the cavity filled with clear lymph fluid.

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55. Denton, M., and Kohman, E.: J. Biol. Chem., 1918, 36, 249.

56. Steenbock, H.: J. Biol. Chem., 1919, 40, 501.

d. Extreme Cases:--The most extreme cases showed an overflow of yellowish liquid from the mouth, making a moist yellow streak from chin to anus, as was earlier observed by Denton.[57]

Just before death the back became very arched, the lower jaw was drawn back and down, and the animals seemed to see poorly even when there was no infection of the conjunctiva. The whole body showed extreme feebleness and emaciation, so that the rats could hardly lift their heads to the top of a two-inch feeding dish. Most of them died in coma, but one, at least, in feeble convulsions.

B. DISCUSSION OF RESULTS.

The results of these experiments seem to justify certain conclusions. In the interpretation of these results, however, allowance must be made for the fact that there were only a few animals on each experimental diet, and that the duration of the experiments was necessarily limited. Nevertheless some deductions may be made, from a consideration of the charts. The deficiency of the sweet potato in adequate proteins and salts, and the adequacy of its vitamines are observations which are in accord with the results obtained by Steenbock, to whose work reference has been made.

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57. Denton, M., and Kohman, E.: J. Biol. Chem., 1918, 36, 249.

VI.

CONCLUSIONS

The results of this series of experiments on the nutritive adequacy of the sweet potato seem to justify the following conclusions.

1. The protein requirement for growth is fulfilled if enough casein be added to the ration to bring the total protein up to 18% of the diet.

2. The nature of the carbohydrates in the sweet potato is not apt to cause intestinal fermentation in rats if the diet is otherwise physiologically correct.

3. A salt mixture, which formed 4% of the total food, supplied sufficient salts for growth, while the sweet potato alone was inadequate in minerals even tho prepared with the skin and therefore the total mineral content included.

4. When 70% to 100% sweet potato was used it supplied fat-soluble-A and water-soluble-B to the diet in fully adequate amounts.

5. Since the sweet potato contains an adequate supply of vitamine, since its carbohydrates are in a highly palatable form, and since it is extensively produced and easy to obtain, it is to be recommended as a general substitute for other starchy foods.

VII.

CHARTS

1. Normal rats, mixed diet, combined curves.
2. Sweet potato diet.
3. Sweet potato to sweet potato, casein diet: to normal diet.
4. Sweet potato, casein diet.
5. Sweet potato, salts diet.
6. Sweet potato, butter diet.
7. Sweet potato, yeast diet.
8. Sweet potato, salts, yeast diet.
9. Sweet potato, salts, butter diet: sweet potato, salts, butter, yeast diet.
10. Sweet potato, butter, yeast diet.
11. Sweet potato, casein, yeast diet.
12. Sweet potato, casein, butter diet.
13. Sweet potato, casein, butter, yeast diet.
14. Sweet potato, casein, salts diet.
15. Sweet potato, casein, salts, butter diet.
16. Sweet potato, casein, salts, butter, yeast diet.
17. Sweet potato, casein, salts, yeast diet.
18. Normal rats.
19. Normal rats.
20. Normal rats.

21. Breeding rats.

22. Breeding rats.

23. Breeding rats.

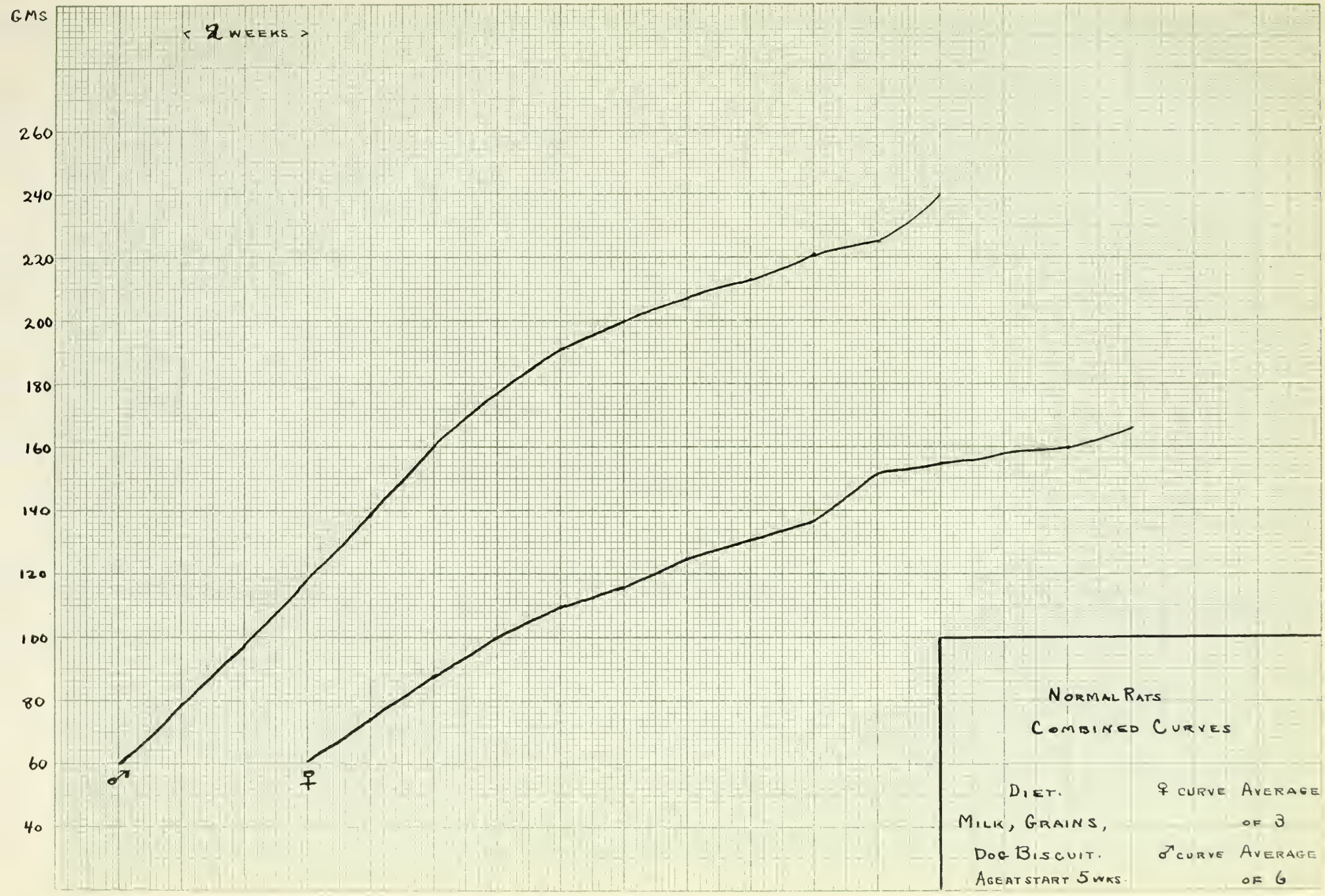

GMS
< 2 WEEKS >
260
240
220
200
180
160
140
120
100
80
60
40
♂
♀
NORMAL RATS
COMBINED CURVES
DIET.
MILK, GRAINS,
DOG BISCUIT.
AGE AT START 5 WKS.
♀ CURVE AVERAGE OF 3
♂ CURVE AVERAGE OF 6

CHART 1

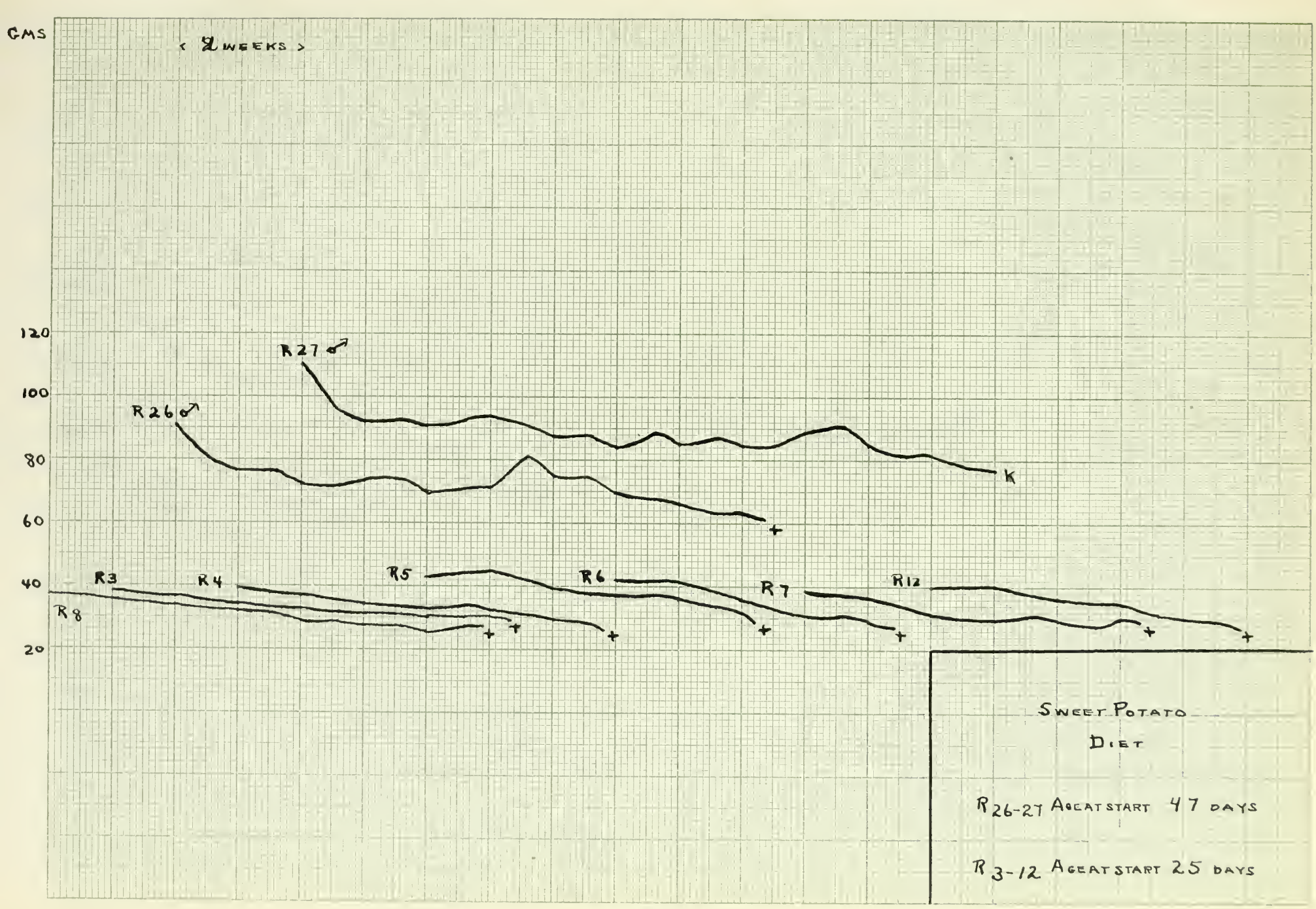

CMS
< 2 WEEKS >
120
100
80
60
40
20
R27 ♂
R26 ♂
R3
R4
R5
R6
R7
R12
R8
K
+
SWEET POTATO
DIET
R26-27 AGE AT START 47 DAYS
R3-12 AGE AT START 25 DAYS

CHART 2

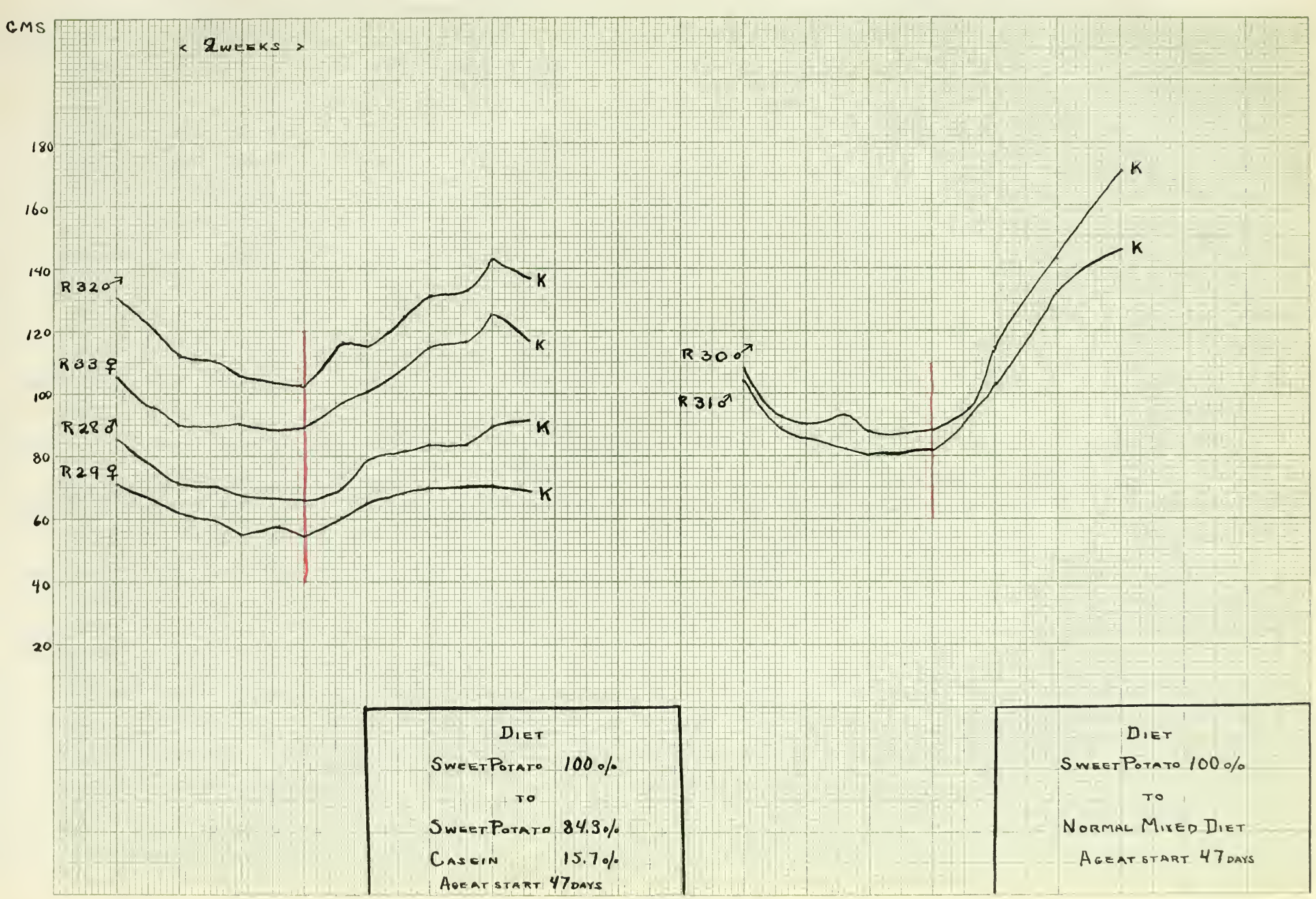

CMS
< 2 WEEKS >
180
160
140
120
100
80
60
40
20
R 32 ♂
R 33 ♀
R 28 ♂
R 29 ♀
K
K
K
K
R 30 ♂
R 31 ♂
K
K
DIET
SWEET POTATO 100 %
TO
SWEET POTATO 84.3 %
CASEIN 15.7 %
AGE AT START 47 DAYS
DIET
SWEET POTATO 100 %
TO
NORMAL MIXED DIET
AGE AT START 47 DAYS

CHART 3

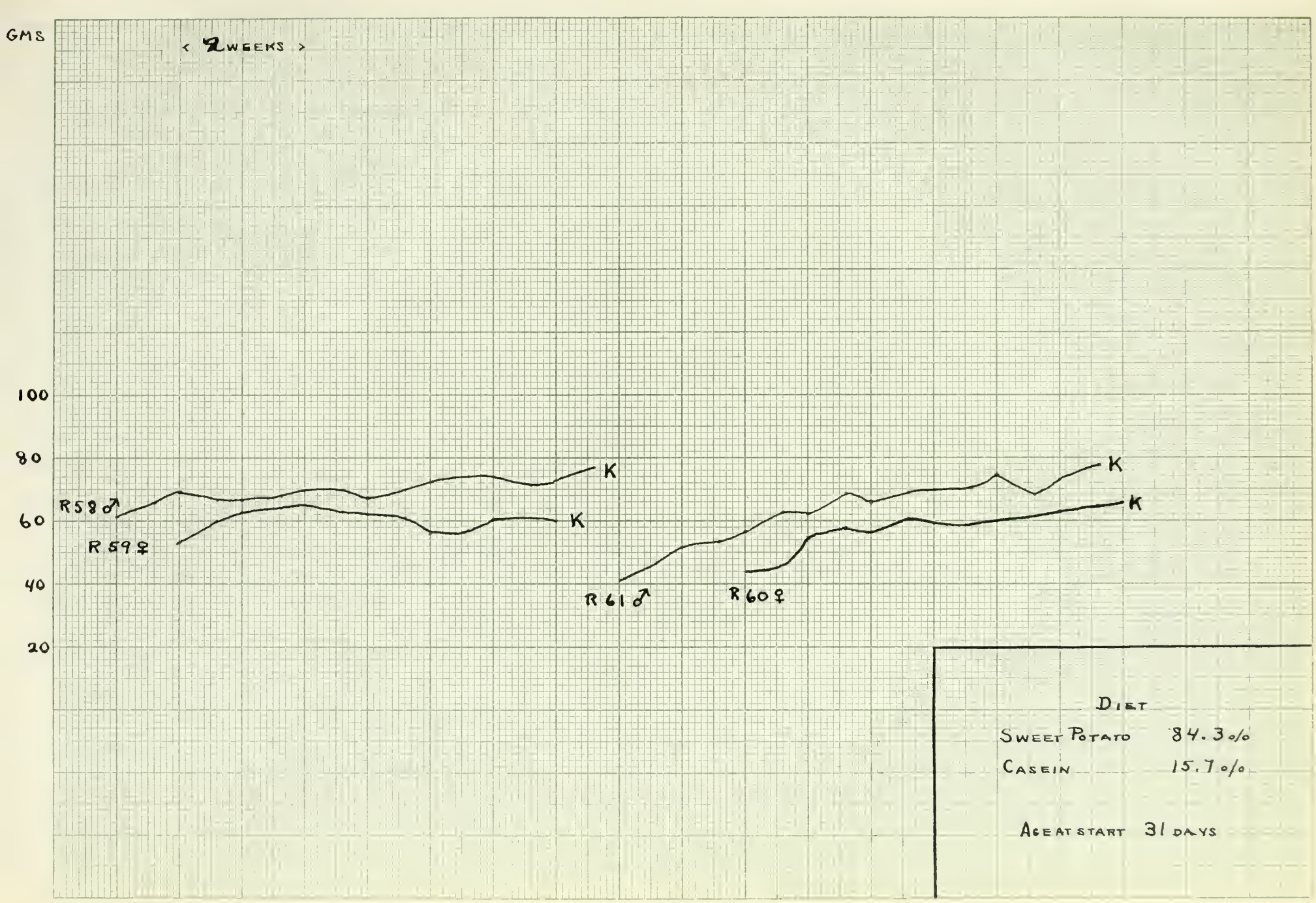

GMS
< 2 WEEKS >
100
80
60
40
20
R58 ♂
R59 ♀
K
K
R61 ♂
R60 ♀
K
K
DIET
SWEET POTATO 84.3%
CASEIN 15.7%
AGE AT START 31 DAYS

CHART 4

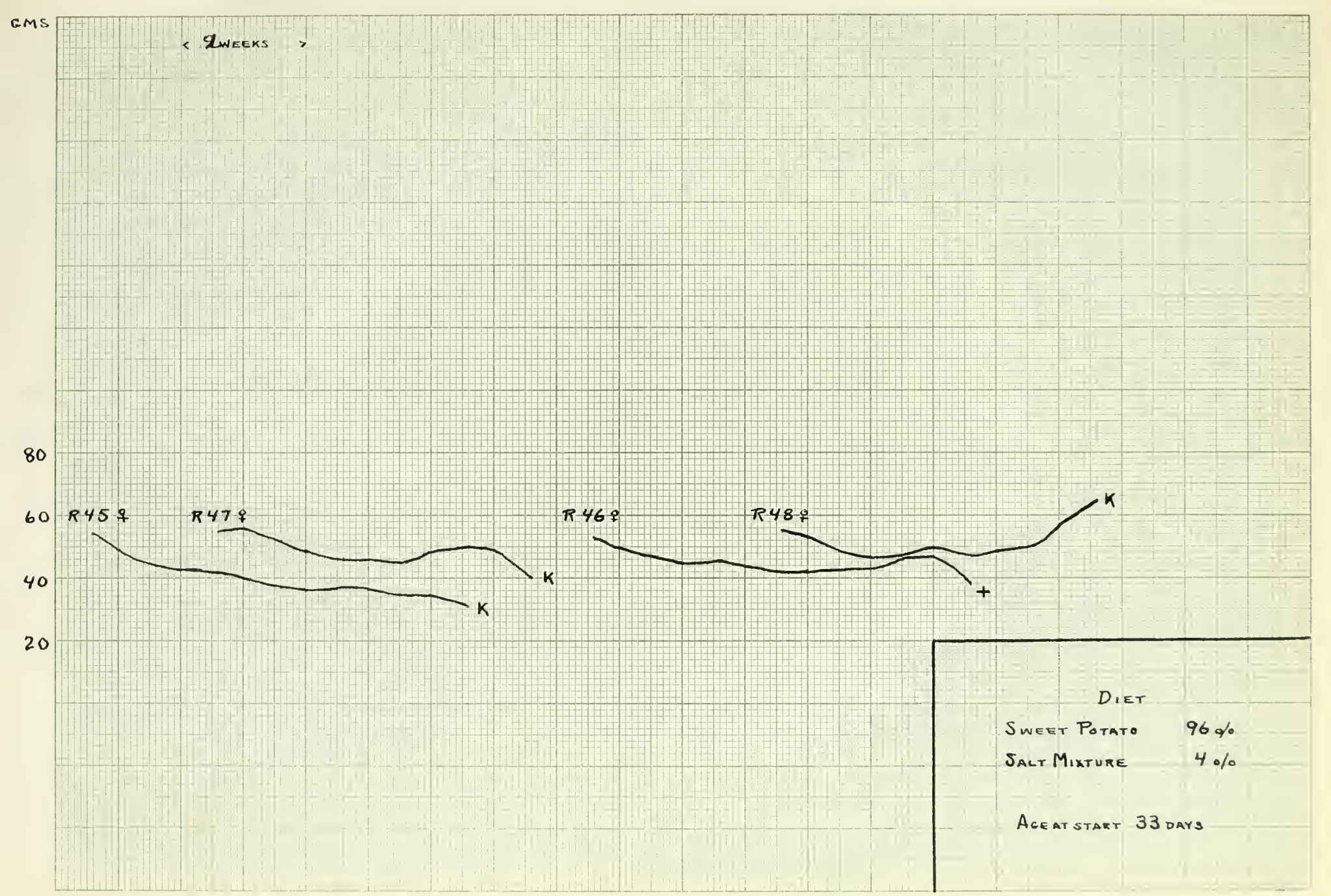

GMS
< 2 WEEKS >
80
60
40
20
R45 ♀
R47 ♀
R46 ♀
R48 ♀
K
K
K
+
DIET
SWEET POTATO 96 o/o
SALT MIXTURE 4 o/o
AGE AT START 33 DAYS

CHART 5

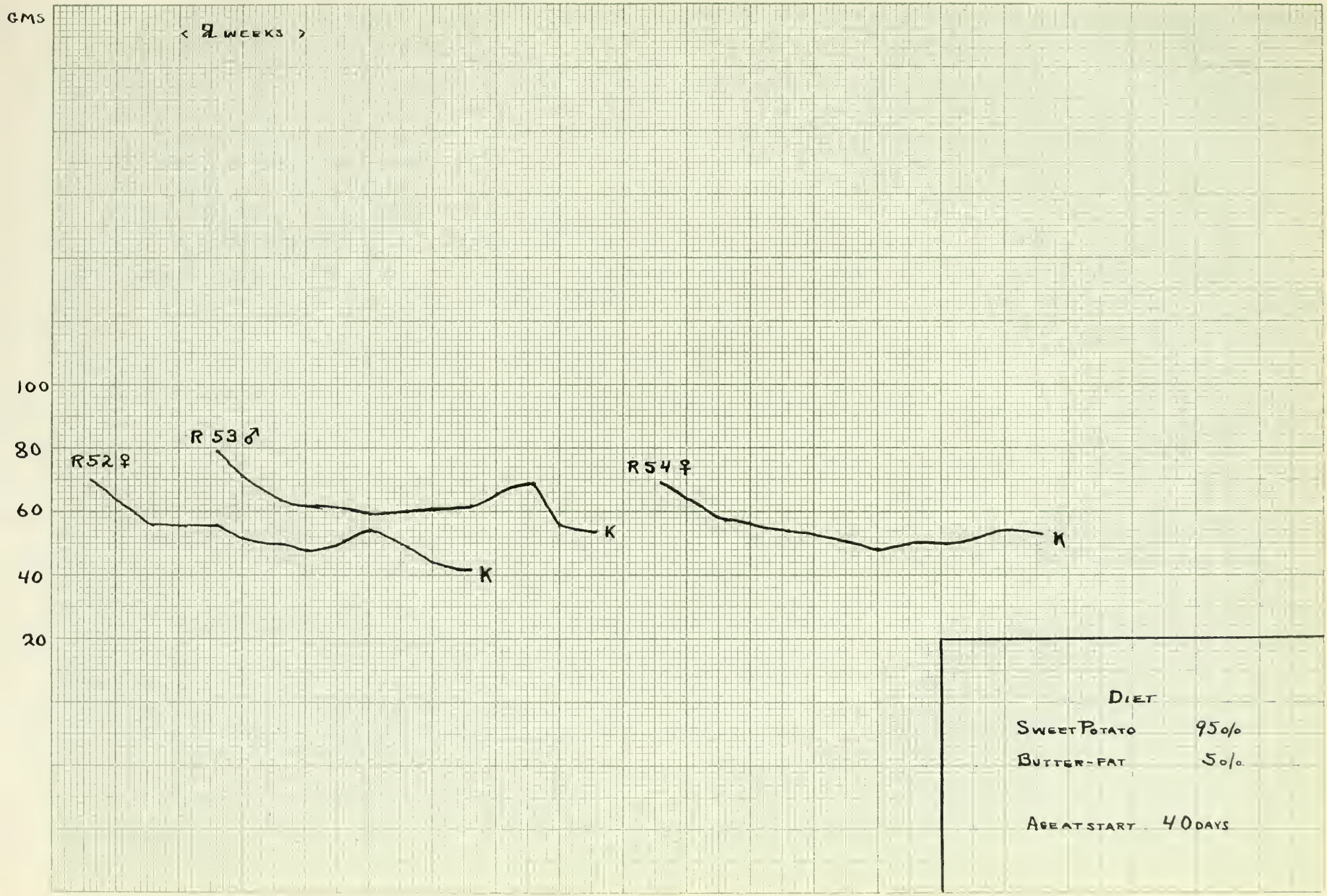

GMS
< 2 WEEKS >
100
80
60
40
20
R52 ♀
R53 ♂
R54 ♀
K
K
K
DIET
SWEET POTATO 95%
BUTTER-FAT 5%
AGE AT START 40 DAYS

CHART 6

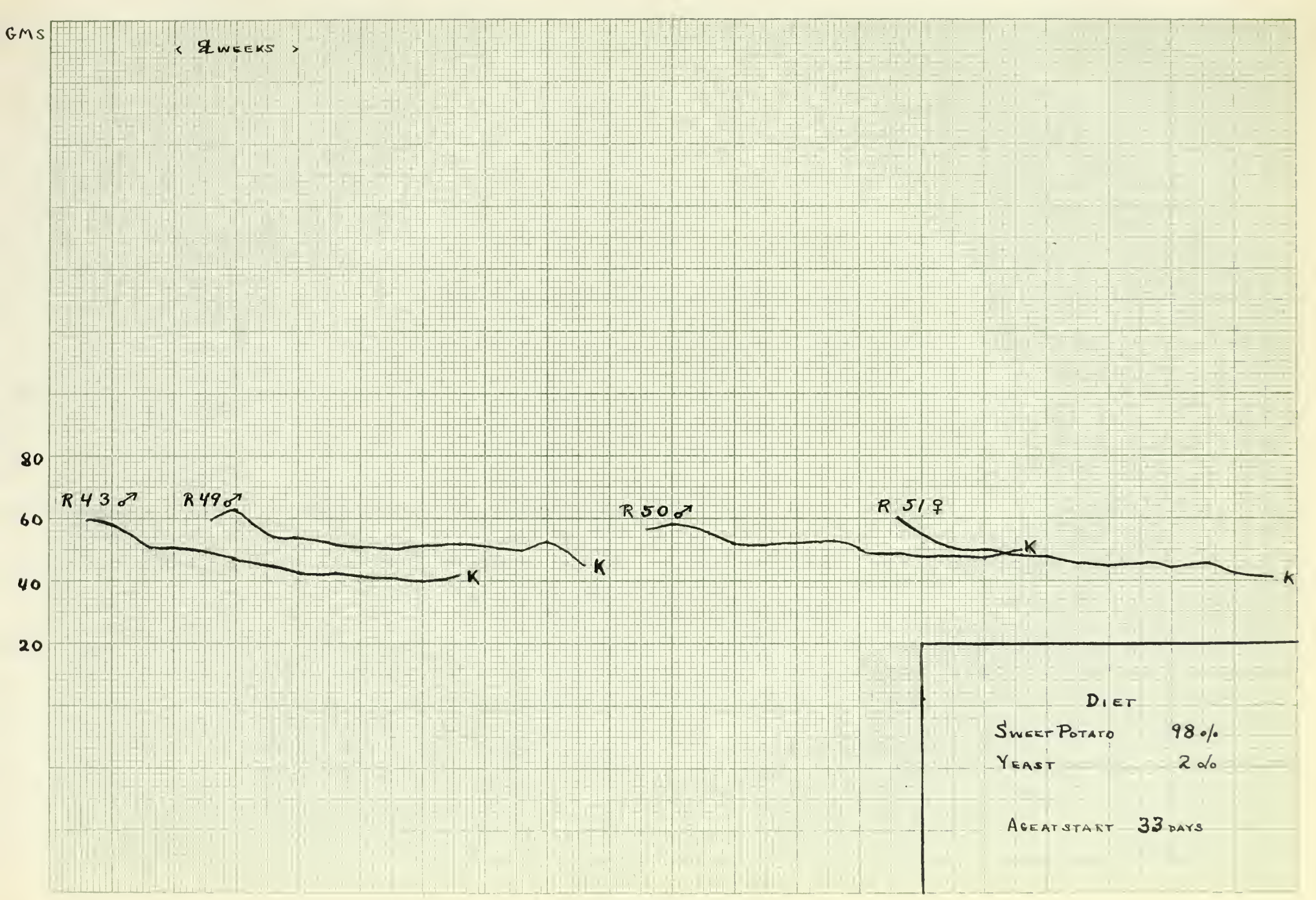

GMS
< 2 WEEKS >
80
60
40
20
R 43 ♂
R 49 ♂
K
K
R 50 ♂
R 51 ♀
K
K
DIET
SWEET POTATO 98 %
YEAST 2 %
AGE AT START 33 DAYS

CHART 7

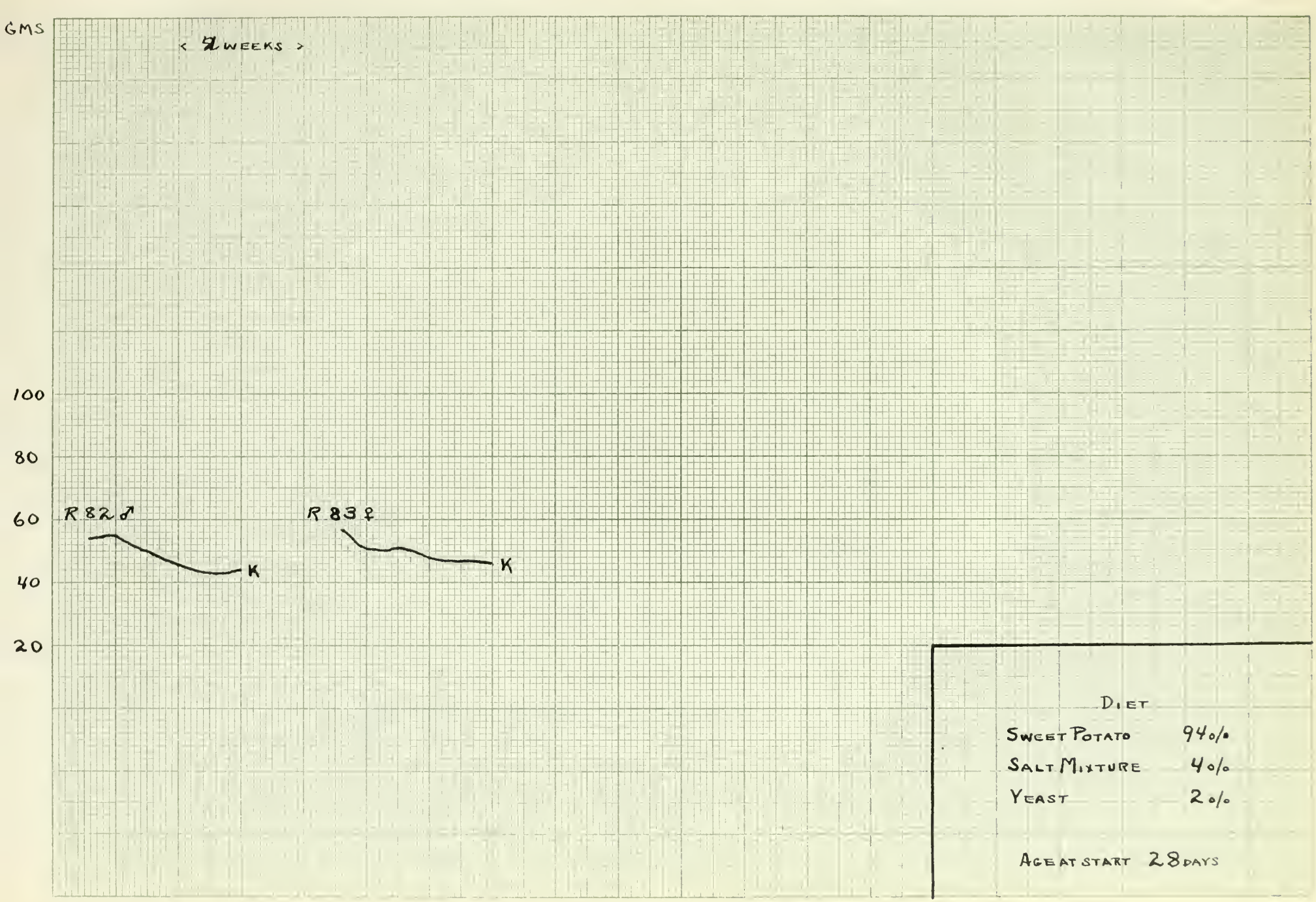
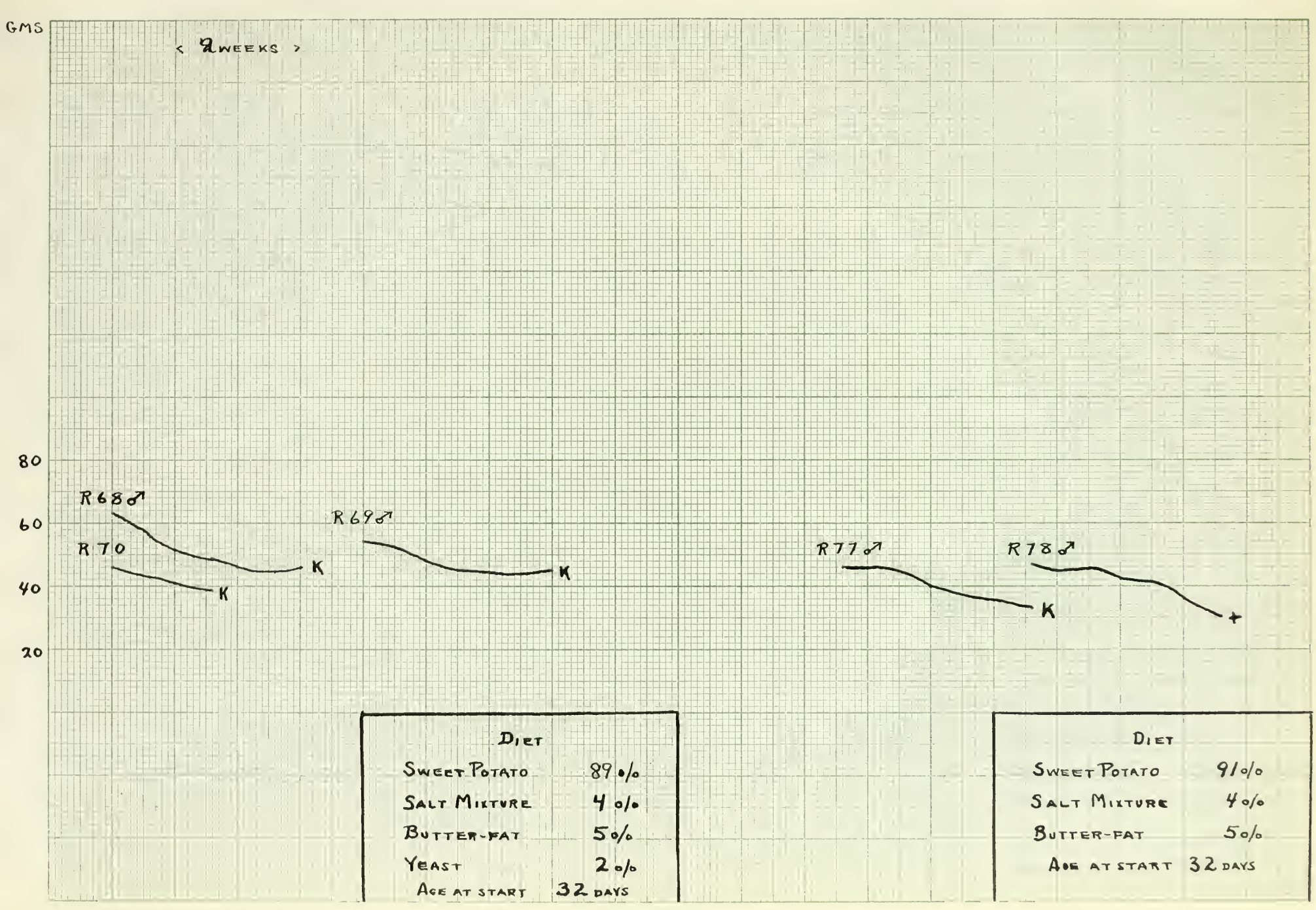

GMS
< 2 WEEKS >
100
80
60
40
20
R 82 ♂
K
R 83 ♀
K
DIET
SWEET POTATO 94%
SALT MIXTURE 4%
YEAST 2%
AGE AT START 28 DAYS

CHART 8



Diet	
Sweet Potato	89 o/o
Salt Mixture	4 o/o
Butter-fat	5 o/o
Yeast	2 o/o
Age at start	32 days

Diet	
Sweet Potato	91 o/o
Salt Mixture	4 o/o
Butter-fat	5 o/o
Age at start	32 days

CHART 9

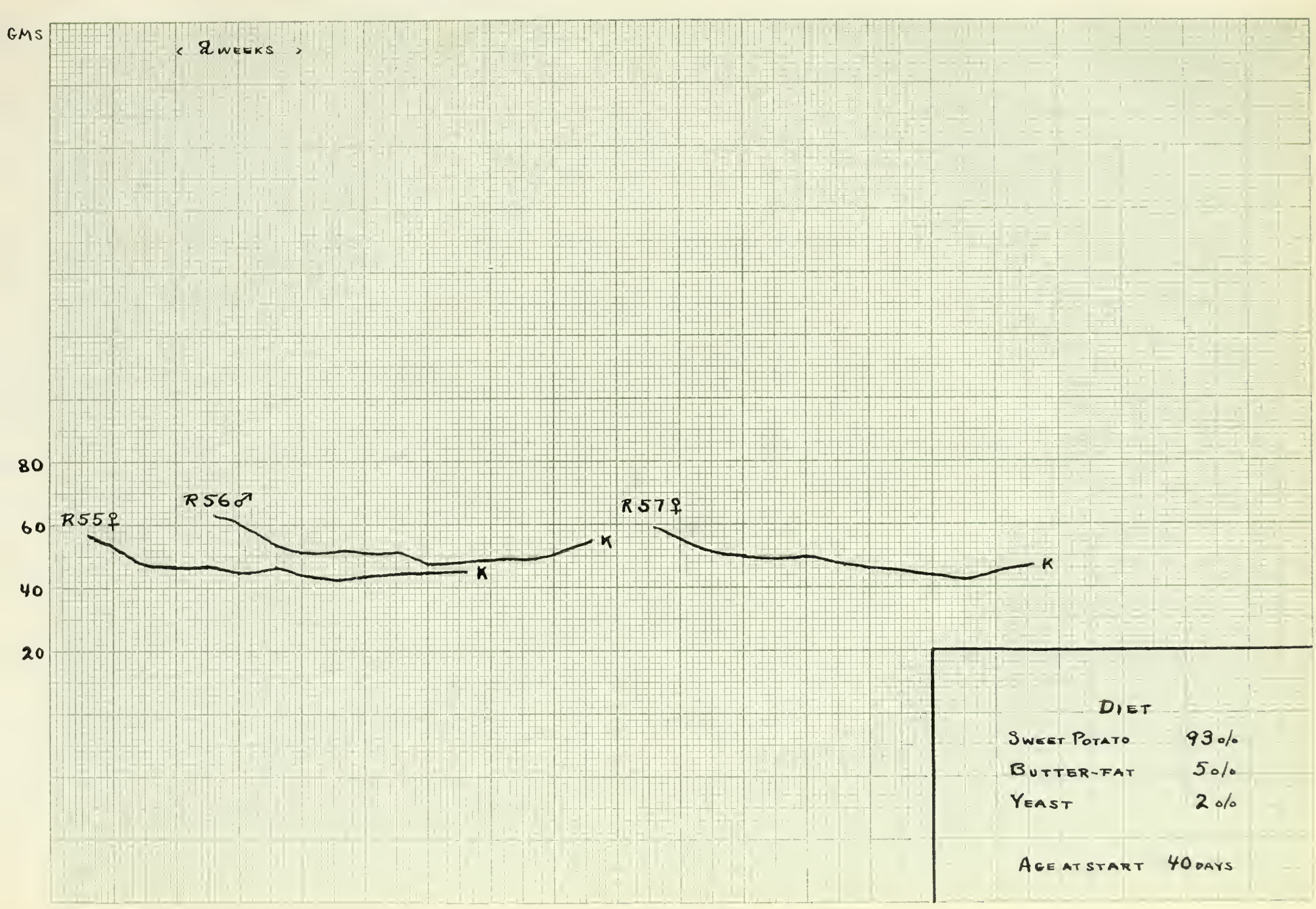

GMS
< 2 WEEKS >
80
60
40
20
R55♀
R56♂
R57♀
K
K
K
DIET
SWEET POTATO 93 o/o
BUTTER-FAT 5 o/o
YEAST 2 o/o
AGE AT START 40 DAYS

CHART 10

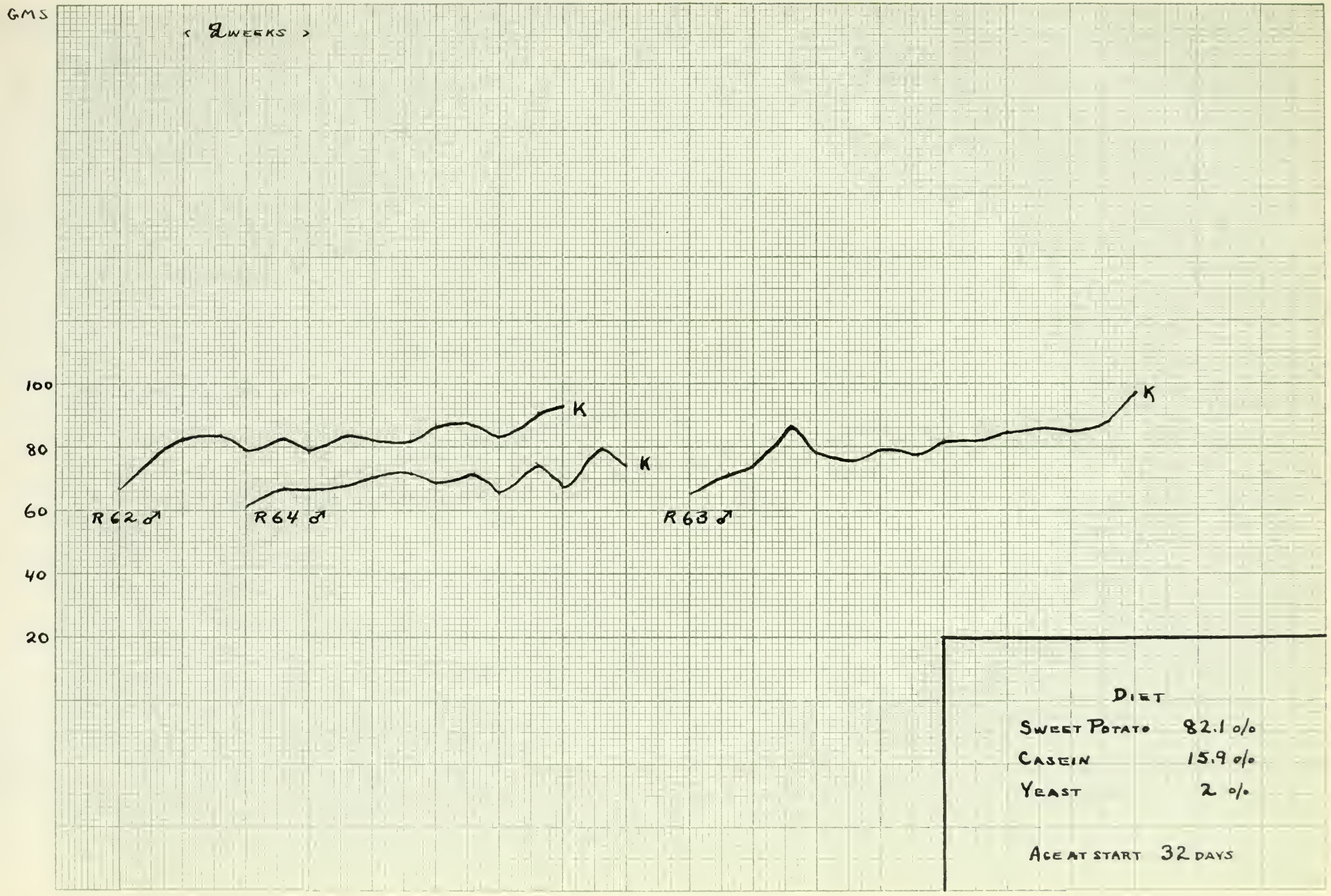

GMS
< 2 WEEKS >
100
80
60
40
20
K
K
K
R 62 ♂
R 64 ♂
R 63 ♂
DIET
SWEET POTATO 82.1 o/o
CASEIN 15.9 o/o
YEAST 2 o/o
AGE AT START 32 DAYS

CHART II

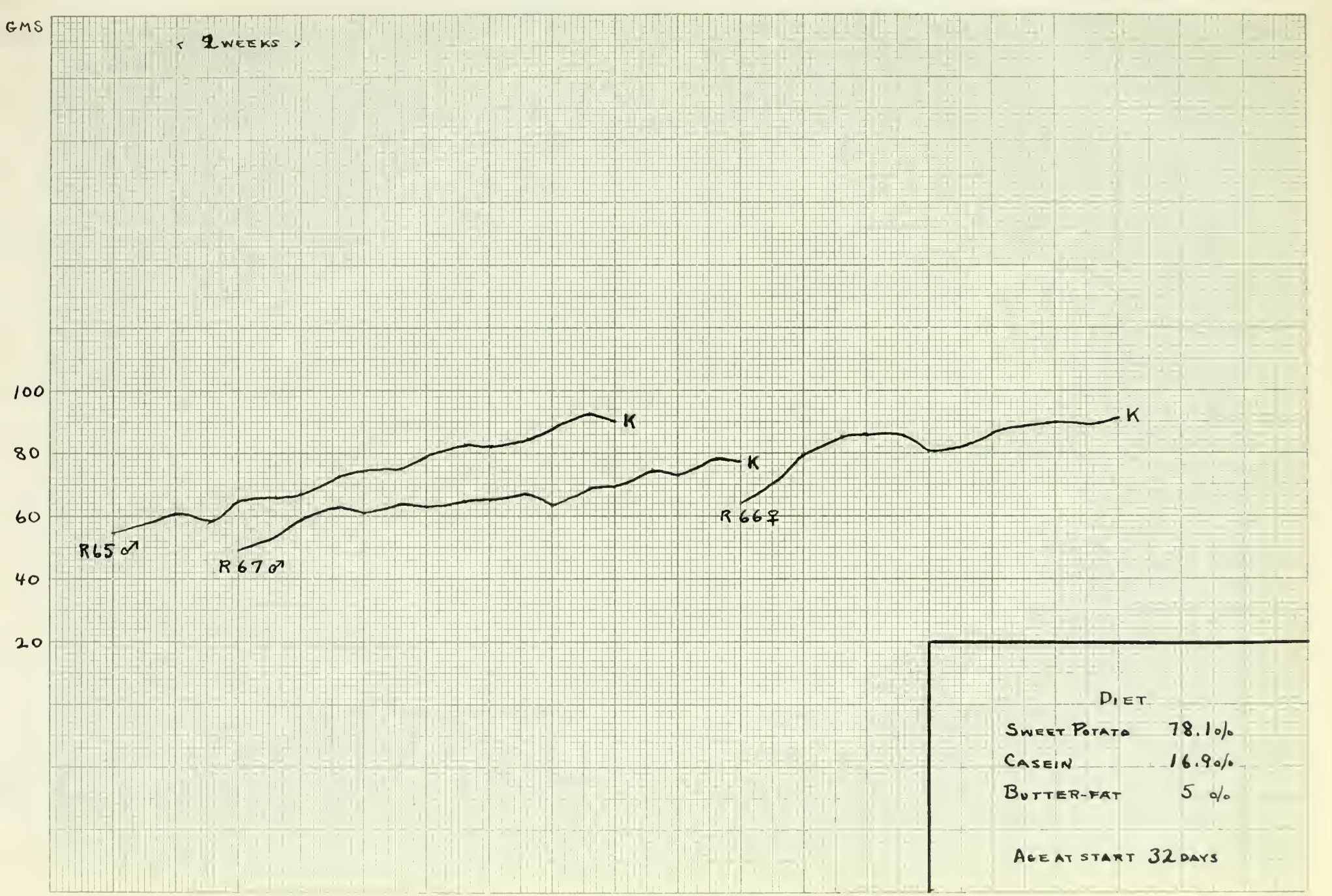

GMS
< 2 WEEKS >
100
80
60
40
20
R65 ♂
R67 ♂
R66 ♀
K
K
K
DIET
SWEET POTATO 78.1%
CASEIN 16.9%
BUTTER-FAT 5 %
AGE AT START 32 DAYS

CHART 12

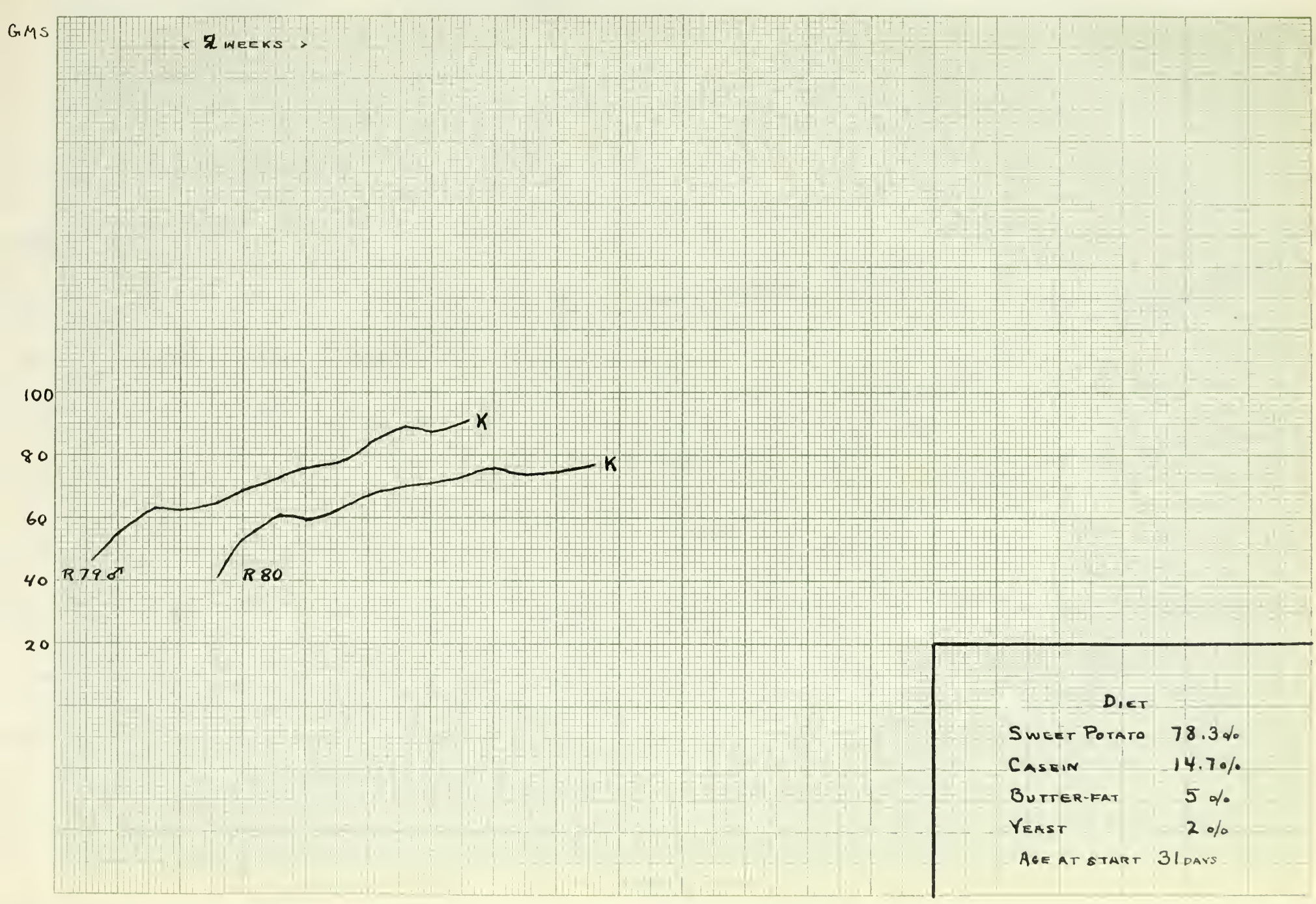

GMS
< 2 WEEKS >
100
80
60
40
20
K
K
R 79 ♂
R 80
DIET
SWEET POTATO 78.3 o/o
CASEIN 14.7 o/o
BUTTER-FAT 5 o/o
YEAST 2 o/o
AGE AT START 31 DAYS

CHART 13

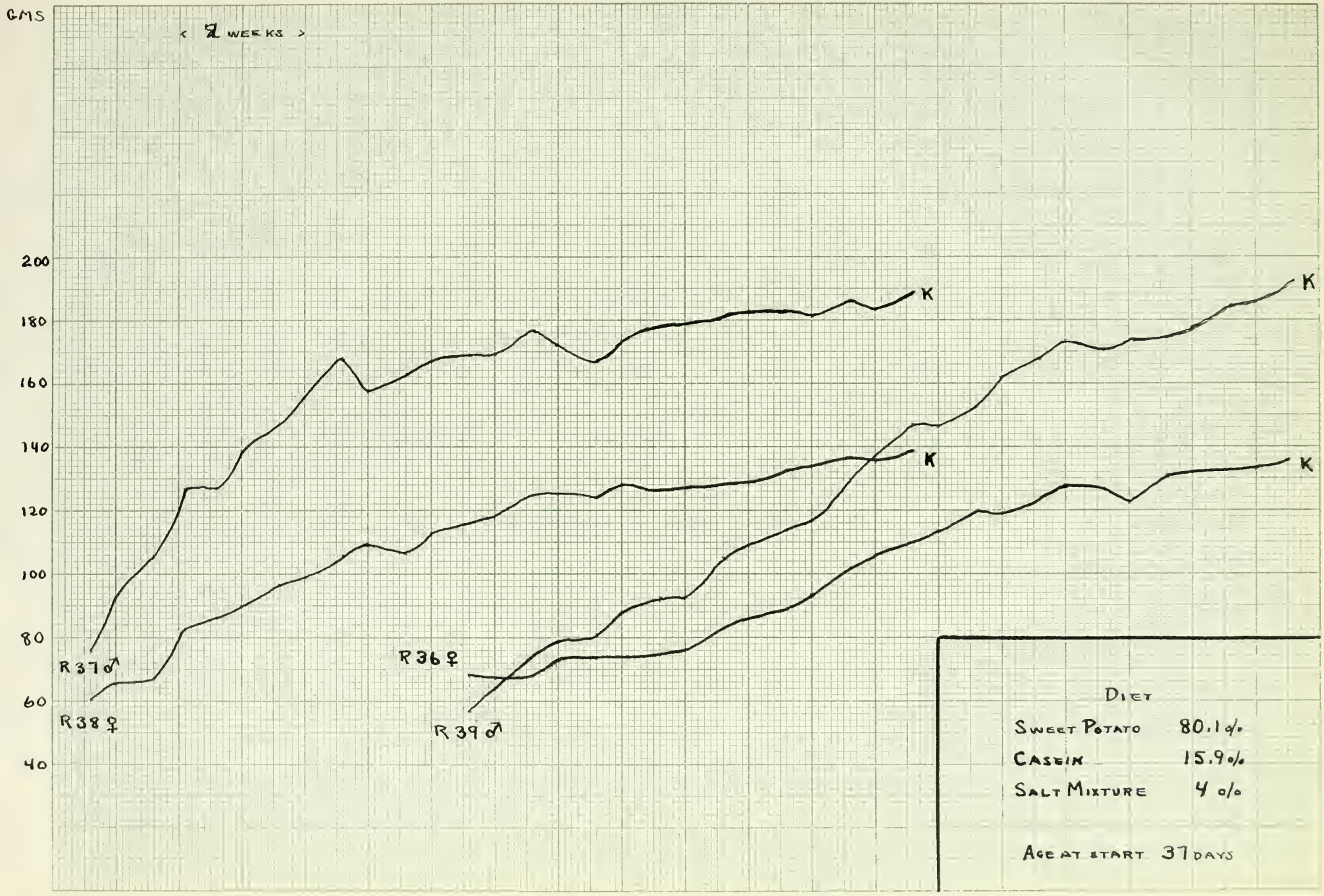

GMS
< 2 WEEKS >
200
180
160
140
120
100
80
60
40
K
K
K
K
R37♂
R38♀
R36♀
R39♂
DIET
SWEET POTATO 80.1%
CASEIN 15.9%
SALT MIXTURE 4 o/o
AGE AT START 37 DAYS

CHART 14

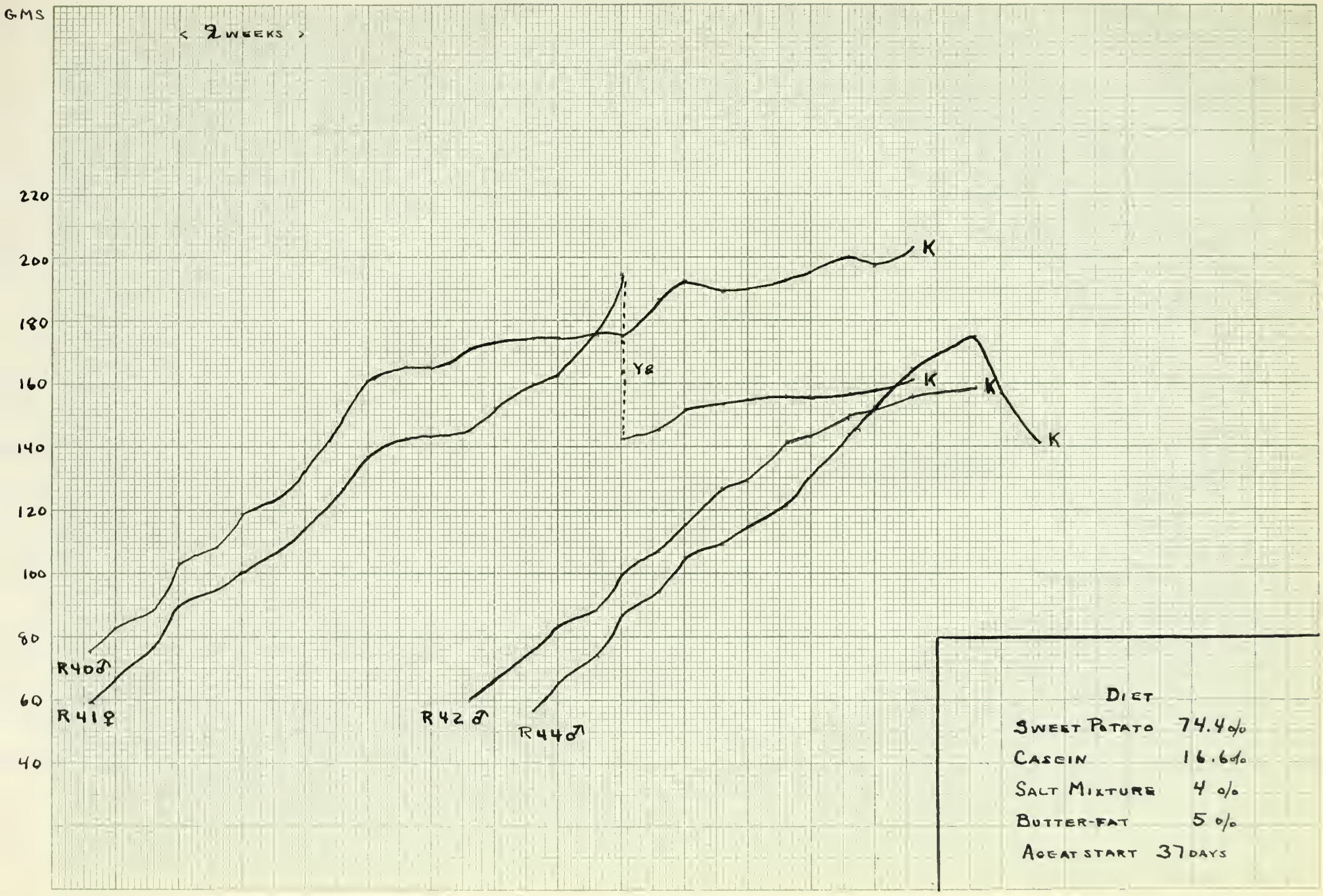

GMS
< 2 WEEKS >
220
200
180
160
140
120
100
80
60
40
K
K
K
K
Y8
R40♂
R41♀
R42♂
R44♂
DIET
SWEET POTATO 74.4%
CASEIN 16.6%
SALT MIXTURE 4 %
BUTTER-FAT 5 %
AGE AT START 37 DAYS

CHART 15

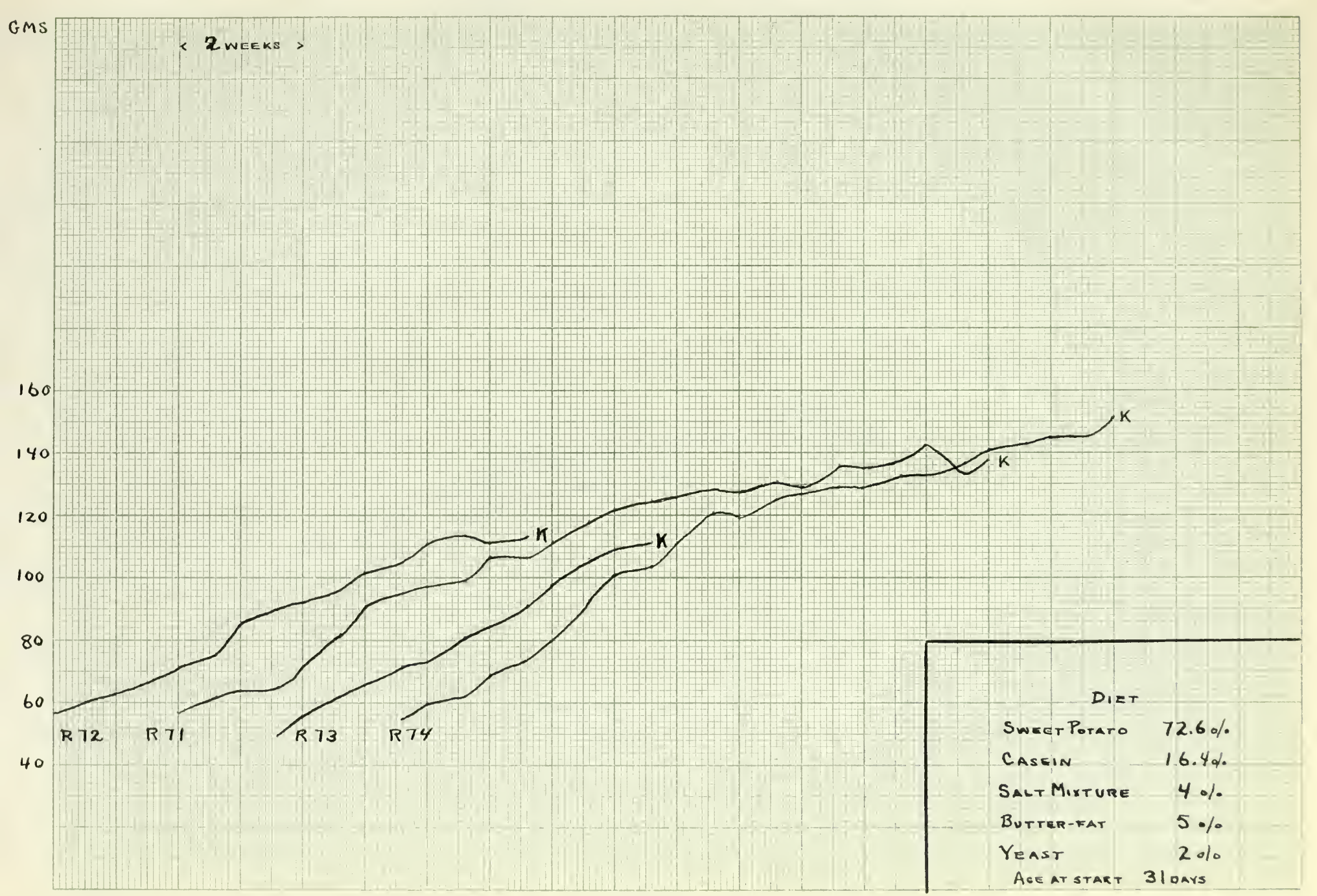

GMS
< 2 WEEKS >
160
140
120
100
80
60
40
R 72
R 71
R 73
R 74
K
K
K
K
DIET
SWEET POTATO 72.6 o/o
CASEIN 16.4 o/o
SALT MIXTURE 4 o/o
BUTTER-FAT 5 o/o
YEAST 2 o/o
AGE AT START 31 DAYS

CHART 16

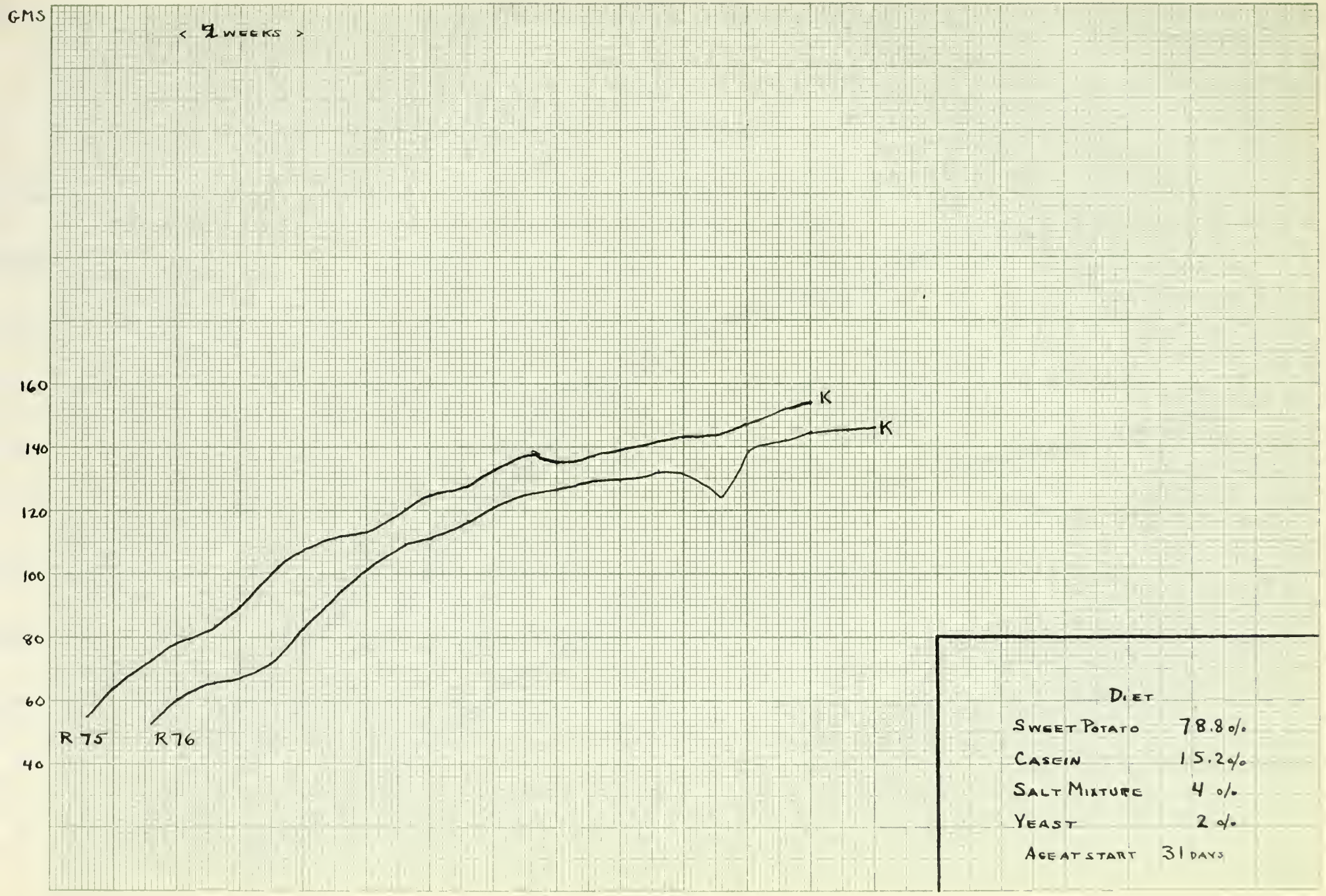

GMS
< 2 WEEKS >
160
140
120
100
80
60
40
K
K
R 75
R 76
DIET
SWEET POTATO 78.8%
CASEIN 15.2%
SALT MIXTURE 4 %
YEAST 2 %
AGE AT START 31 DAYS

CHART 17

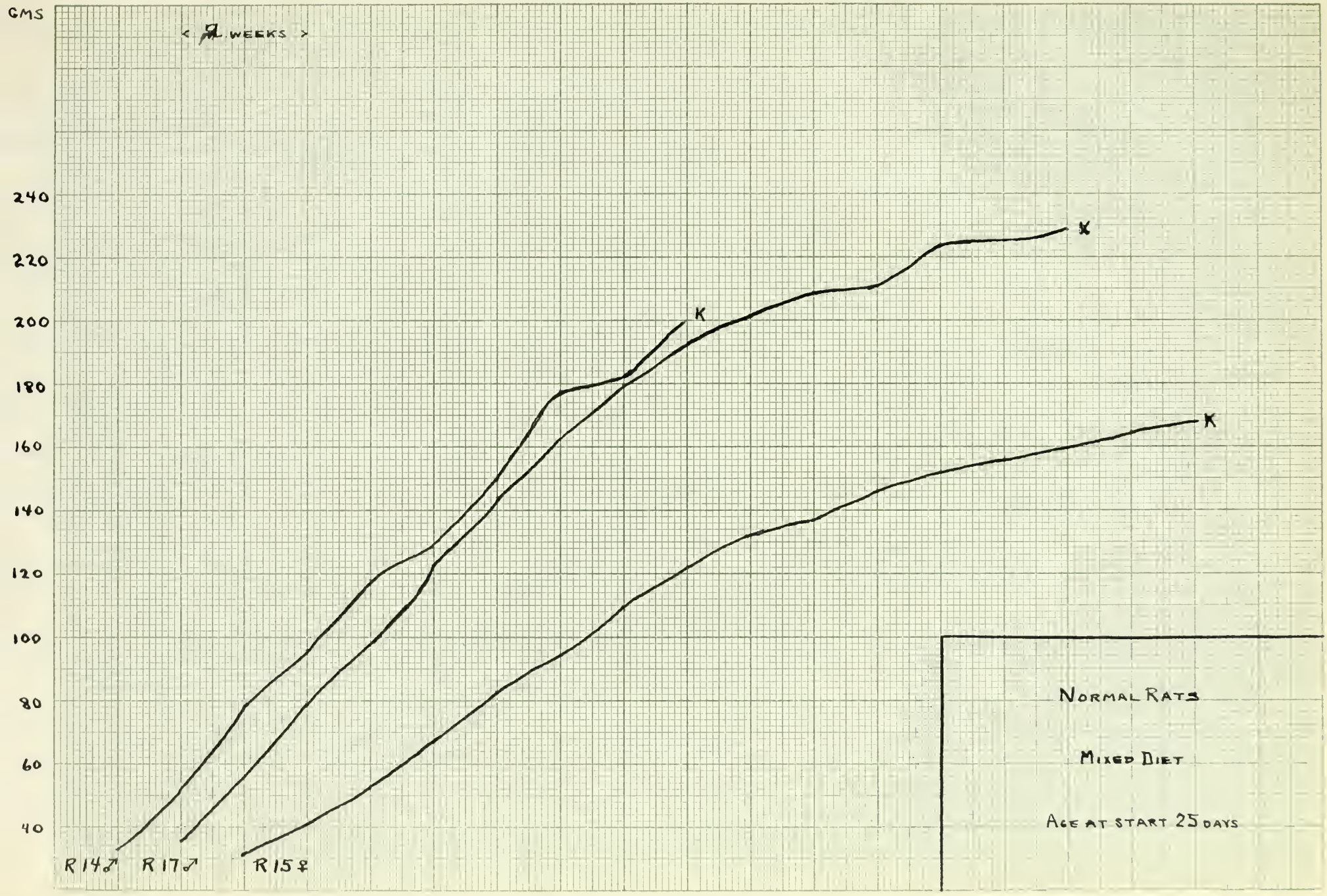

GMS
< 8 WEEKS >
240
220
200
180
160
140
120
100
80
60
40
K
K
K
R 14 ♂
R 17 ♂
R 15 ♀
NORMAL RATS
MIXED DIET
AGE AT START 25 DAYS

CHART 18

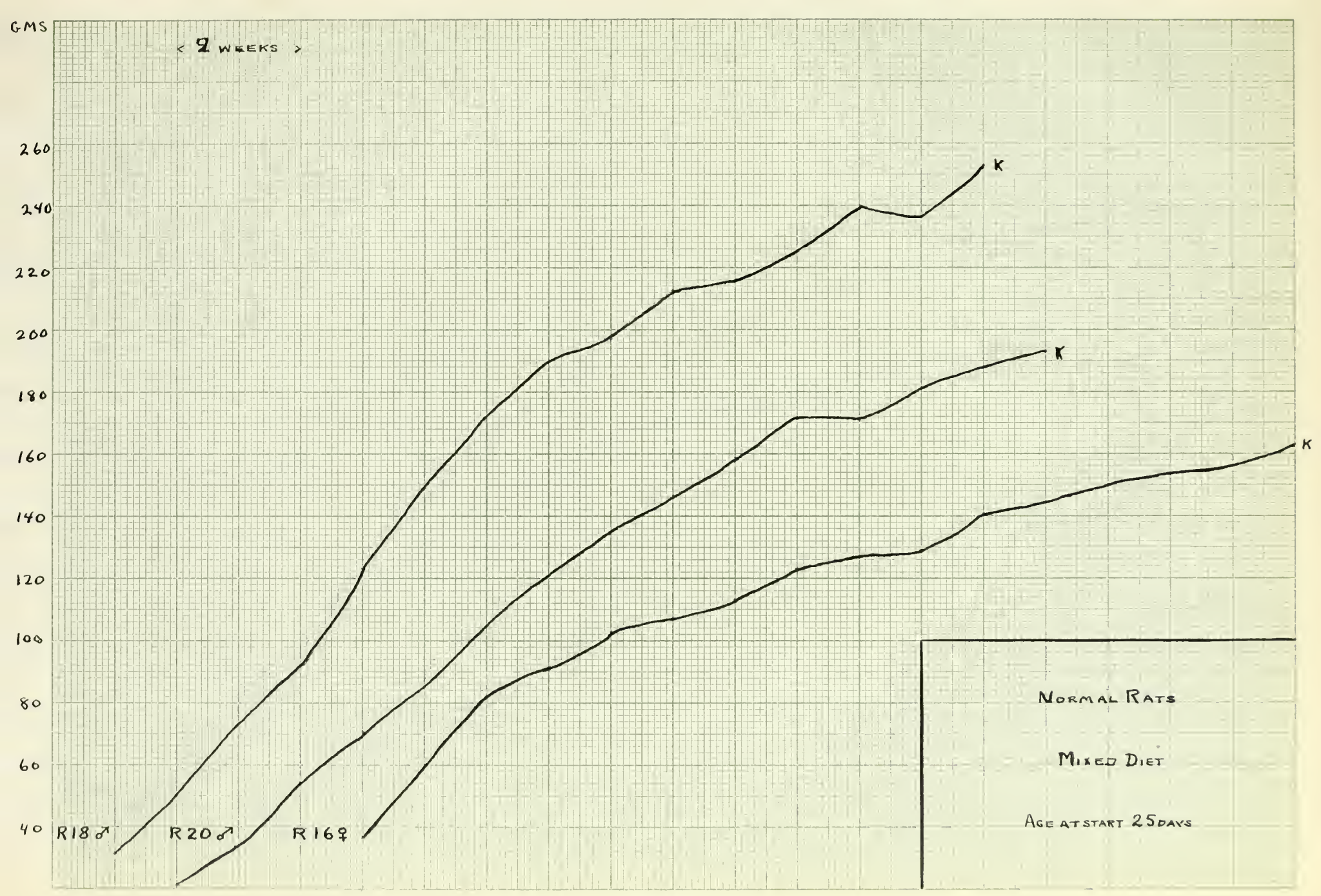

GMS
< 9 WEEKS >
260
240
220
200
180
160
140
120
100
80
60
40
K
K
K
R18 ♂
R20 ♂
R16 ♀
NORMAL RATS
MIXED DIET
AGE AT START 25 DAYS

CHART 19

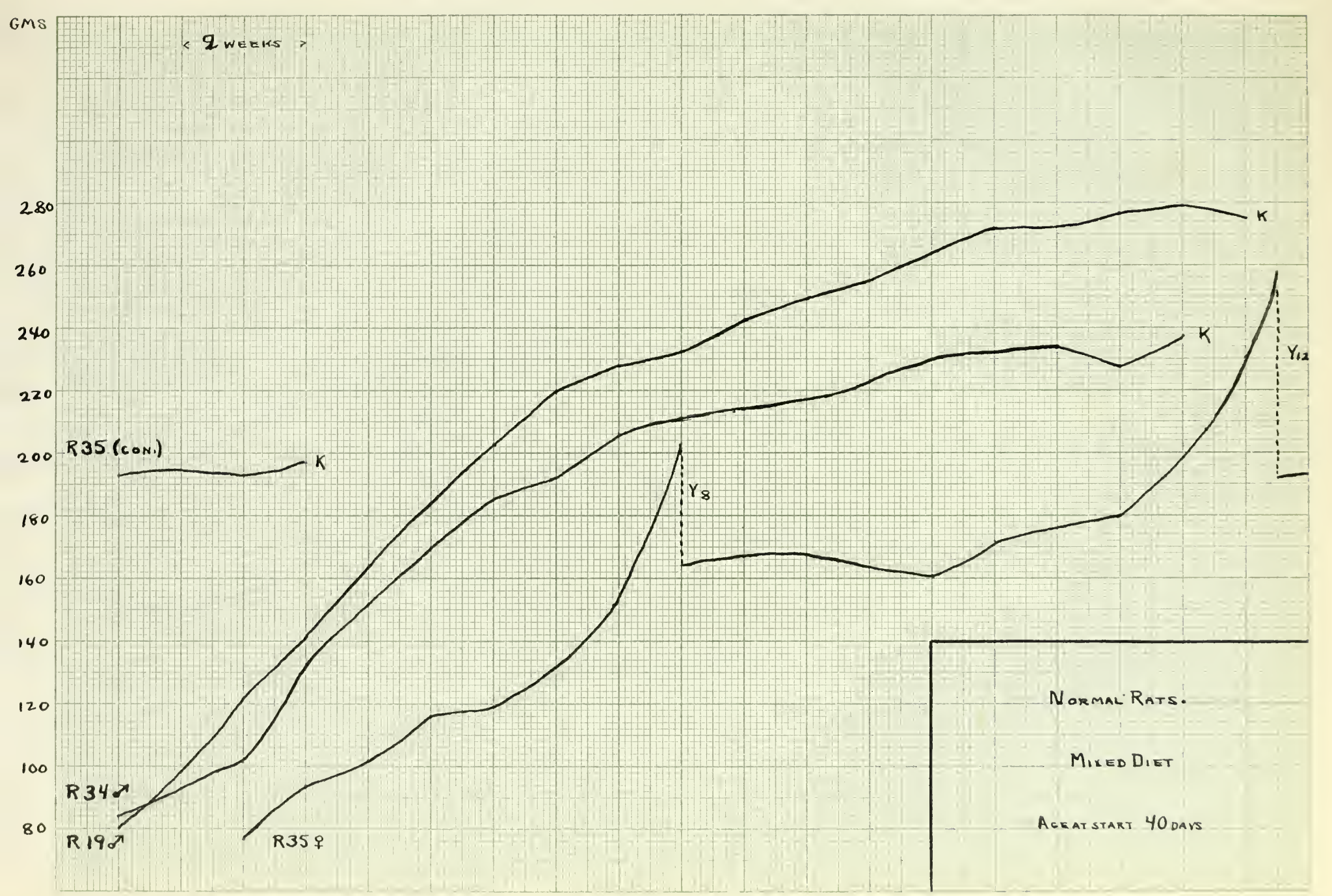

GMS
< 2 WEEKS >
280
260
240
220
200
180
160
140
120
100
80
R35 (CON.)
K
K
K
R34♂
R19♂
R35♀
Y8
Y12
NORMAL RATS.
MIXED DIET
AGE AT START 40 DAYS

CHART 20

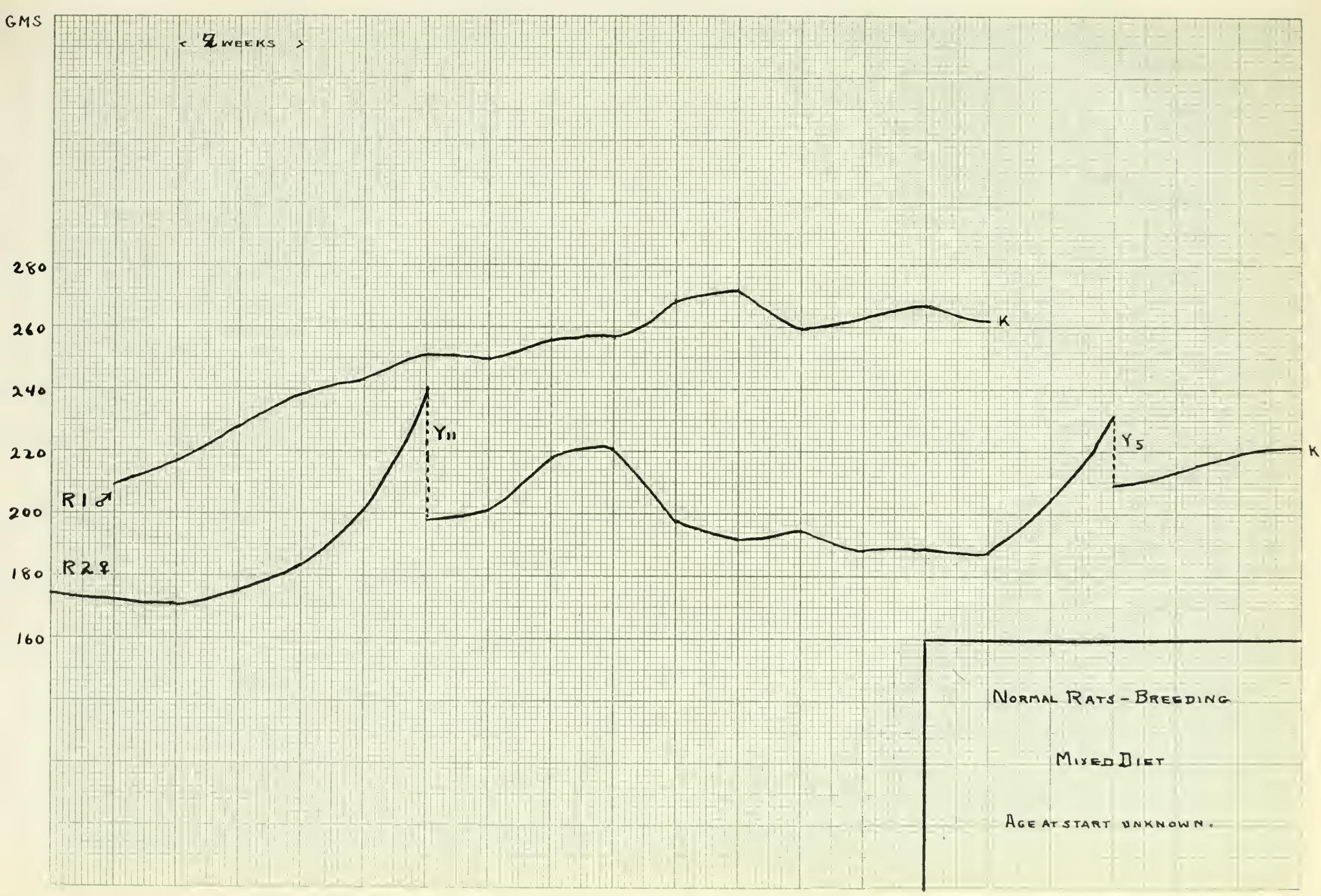

GMS
< 2 WEEKS >
280
260
240
220
200
180
160
K
Y_{11}
Y_5
K
R1 ♂
R2 ♀
NORMAL RATS - BREEDING
MIXED DIET
AGE AT START UNKNOWN.

CHART 21

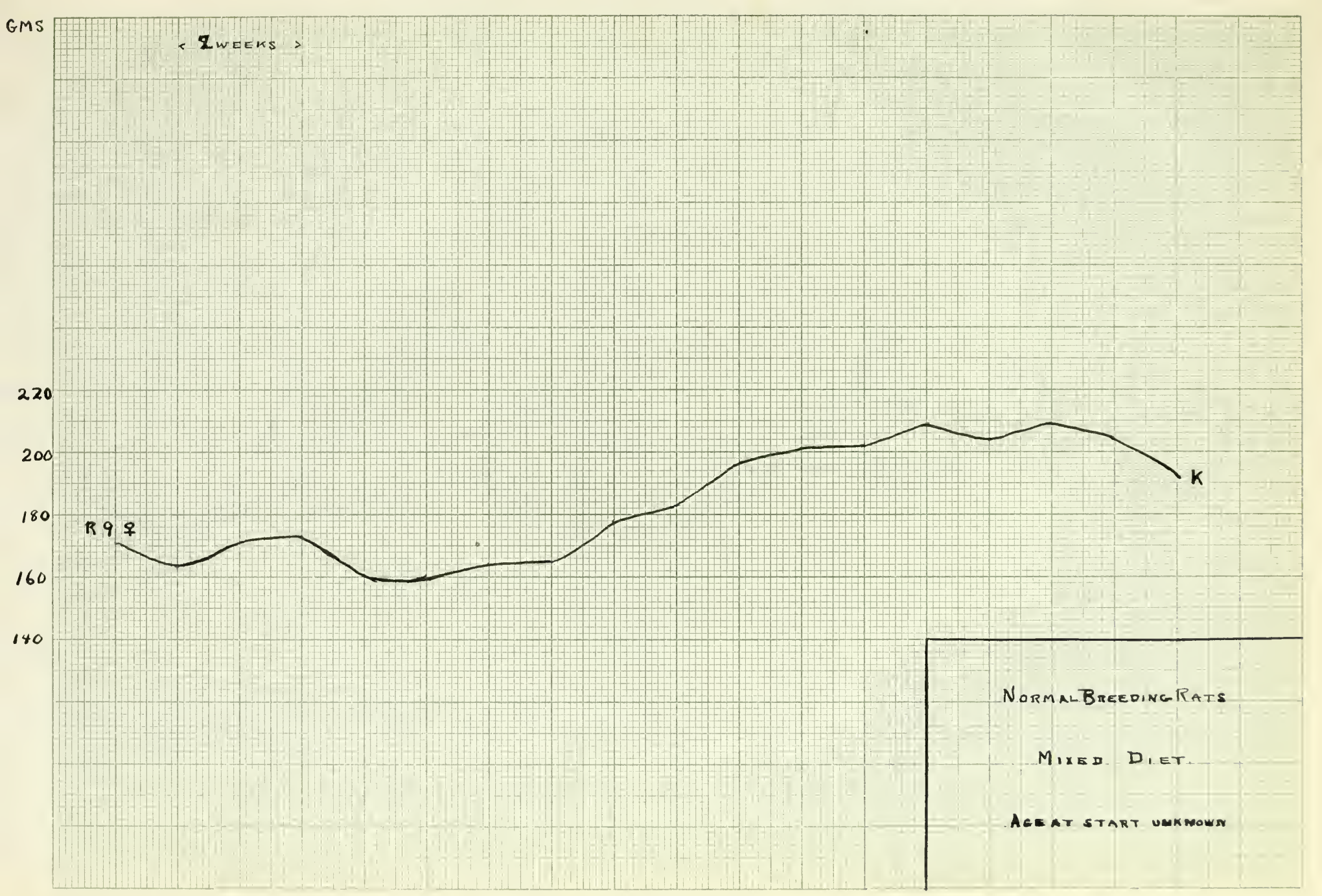

GMS
< 2 WEEKS >
220
200
180
160
140
R 9 ♀
K
NORMAL BREEDING RATS
MIXED DIET
AGE AT START UNKNOWN

CHART 22

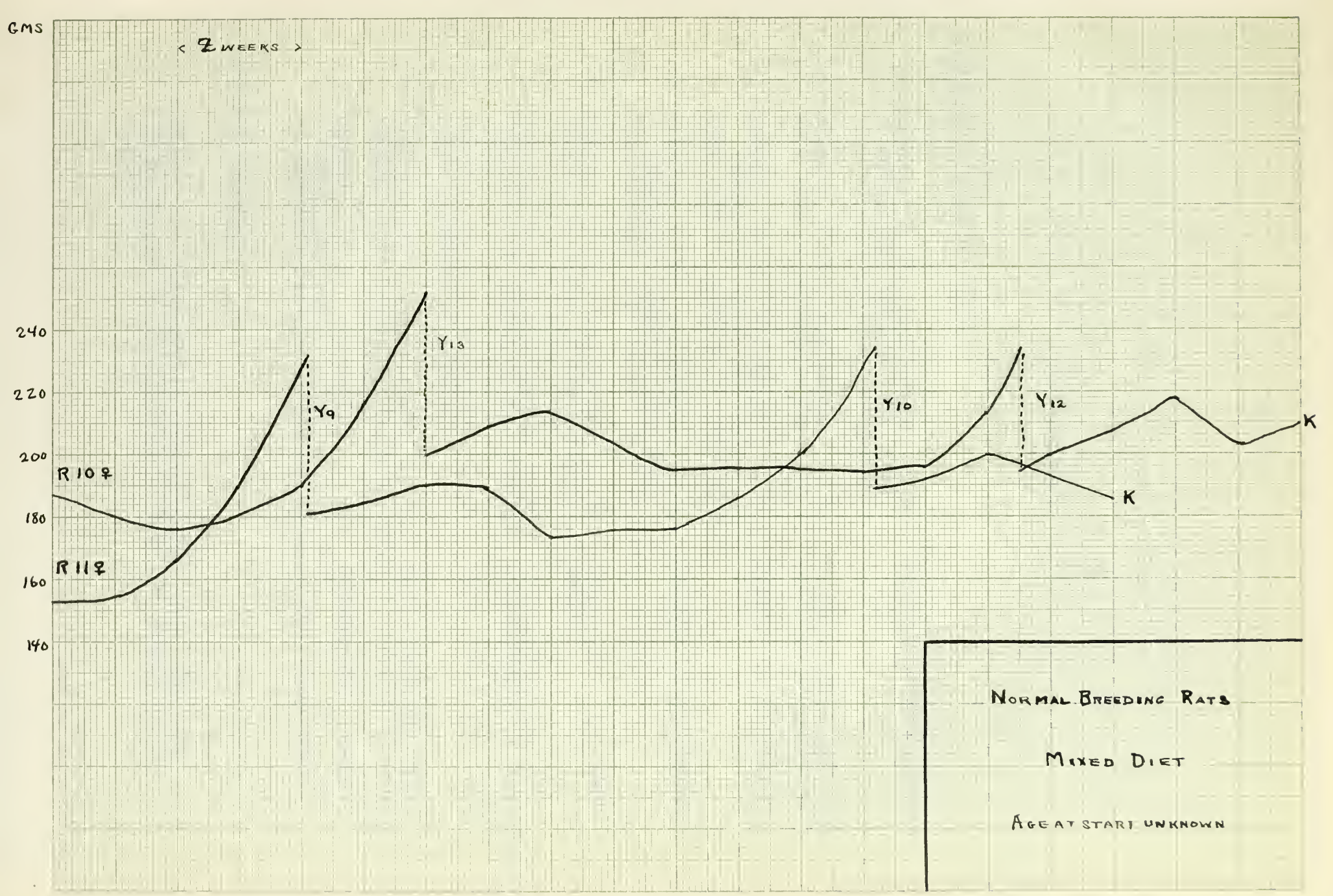

GMS
< 2 WEEKS >
240
220
200
180
160
140
R 10 ♀
R 11 ♀
Y9
Y13
Y10
Y12
K
K
NORMAL BREEDING RATS
MIXED DIET
AGE AT START UNKNOWN

CHART 23

VIII.

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